2017

EVALUATION OF PYRIPROXYFEN APPLIED IN BARRIER SPRAYS FOR MOSQUITO SUPPRESSION

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Digital Object Identifier: https://doi.org/10.13023/ETD.2017.183

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EVALUATION OF PYRIPROXYFEN APPLIED IN BARRIER SPRAYS FOR MOSQUITO SUPPRESSION.

THESIS

A thesis submitted in partial fulfillment of the

requirements for the degree of Master of Science in the

College Agriculture, Food and Environment

at the University of Kentucky

By

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Lexington, Kentucky

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2017

ABSTRACT OF THESIS

EVALUATION OF PYRIPROXYFEN APPLIED IN BARRIER SPRAYS FOR MOSQUITO SUPPRESSION.

Despite advances in mosquito management, mosquito-borne disease in the United States is still of relevant public health concern and vector control is a top priority in preventing transmission of pathogens. Insecticide barrier sprays have become a common tool for suppression of mosquitoes in single-homeowner backyards. The application of the synthetic pyrethroid, lambda-cyhalothrin, to perimeter vegetation with a backpack sprayer has been shown to significantly suppress mosquito levels for around 6 weeks. In an attempt to lengthen the effective duration of treatment, the IGR, pyriproxyfen, was added to a backpack mist blower with lambda-cyhalothrin, as adult mosquitoes exposed to pyriproxyfen have been shown to disseminate it to oviposition sites and to experience lowered fecundity. This treatment was compared to lambda-cyhalothrin alone and to a water control. Mosquito populations were sampled using CO_2-baited CDC light traps, CDC gravid traps, human landing rates, and ovitraps. Leaf bioassays were performed. The following summer, the same treatments were applied with a truck-mounted mist blower to tree lines in Central Kentucky, to test the efficacy of an application method that could be used on large properties. Finally, bioassays were performed with water sampled from pyriproxyfen-treated containers, exposed to field conditions to test for residual efficacy.

KEYWORDS: pyriproxyfen; insect growth regulator; lambda-cyhalothrin; barrier; *Aedes*; *Culex*

Andrea Glenn Skiles

May 5, 2017

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May 5, 2017

“I suppose if we couldn’t laugh at things that don’t make sense, we couldn’t react to a lot of life.” Bill Watterson

For my father, David L. Skiles

ACKNOWLEDGMENTS

I would like to thank first and foremost, my thesis advisor, Dr. Grayson C. Brown, for taking me into his lab and giving me the opportunity to pursue Public Health Entomology. He has been incredibly supportive of my involvement in many career-building experiences, particularly when it has come to participating in professional societies and these have been greatly fulfilling. I look forward to many more years of collaboration.

Next I would like to thank the members of my committee, Dr. Michael F. Potter and Dr. John J. Obrycki for their guidance in completing this thesis and helping to make it the best that it can be. They have provided valuable perspectives and their time is greatly appreciated. The Department of Entomology faculty and staff have also served as a vast source of knowledge. Those whose instruction I could not have developed as a researcher without include: Dr. Kenneth Haynes, Dr. Daniel Potter, Dr. Jen White, Dr. Lee Townsend, and Dr. Eric Chapman.

Funding for this project was secured by our Syngenta representative, Dr. Nicola Gallagher, who was also instrumental in developing this research. As anyone who has conducted fieldwork knows, it is not accomplished without the cooperation and labor of many people, and for this project they were: Caitlin Stamper, Caleb Maas, Paul Bellamy, and Rebecca Brown. Your sweat is remembered.

I will leave Kentucky with many memories and some of the best will include my comrades in the Department of Entomology, particularly: Jennifer Gordon, Sydney Crawley, Kelly Jackson, Paul Lenhart, Bill Davidson, Matt Savage, Abe Nielson, and Allison Dehnel. I must give special thanks to my lab-mate, close friend, and superstar, Kyndall Braumuller, for all of her help and inspiration.

Finally, I thank my friends and family: Kayla DeChaine and Lauren Manninen, for being the sisters I got to choose; my mother, Stacy, who has always viewed my potential as limitless; my aunts, uncles, and cousins, who have never let any claim I've ever made go unchallenged, having unwittingly prepared me well for a career in science; my siblings Hayley and Jared, who always make me proud; Major, for his unending loyalty; and perhaps most of all, my great-aunt Peg and late grandma Jean – I would have achieved nothing without the support they have provided and their influence has been paramount in forming me into the woman I am today. I could not love you all more.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LITERATURE REVIEW

Public Health Concern of Mosquitoes. Mosquito-borne disease is often considered an issue primarily of tropical importance, particularly among developing countries. Worldwide, many of the worst mosquito-borne diseases are resurging and the combined impact of diseases such as dengue, yellow fever, and chikungunya reach over 400-600 million cases each year (Tolle, M., 2009). The United States is also affected by mosquito-borne disease, however, the disease of chief concern is West Nile Virus (WNV) which accounts for 95% of domestic arboviral disease cases (Krow-Lucal et al., 2017). La Crosse virus, Jamestown Canyon virus, St. Louis encephalitis, and Eastern Equine encephalitis are others found throughout the United States. The Zika virus is emerging as another disease that may have the potential to cause serious public health problems in the United States. Of veterinary concern, dog-heartworm cases are a costly threat to pets exposed to mosquitoes vectoring *Dirofilaria immitis.*

WNV first appeared in the Unites States in 1999 and while the number of cases has lessened in recent years, due to immunity among the bird population, it is still active in all 48 contiguous states (Meek, 2002). This virus is the leading cause of neuroinvasive disease (meningitis, encephalitis, and acute flaccid paralysis) in the United States, and the outbreak here has resulted in considerably more cases than outbreaks in Europe (Voelker et al, 2014; Kilpatrick et al, 2006). It is one of the relatively few mosquito-borne diseases to be found in the northern United States and Canada. Currently, there is no human vaccine for WNV, but equine vaccines do exist. The virus has a bird-human-bird transmission cycle, and mammals only serve as a dead-end host. This means that while WNV can still cause potentially deadly neuroinvasive disease, the viremia of human hosts will not become high enough for WNV to be picked up and passed to mosquitoes feeding on them (Peterson, 2013). This is relevant to future control efforts of WNV, because even if a vaccine were to be developed, it would not contribute to decreasing the viremia in an area, as humans would not transfer the virus to mosquitoes regardless of vaccination status. Instead disease control efforts are focused in large part on decreasing the vector populations in areas where human and animal risk of exposure is greatest. In northern California, researchers presented evidence that preventative truck and aerial ultra-low volume sprays of adulticide effectively reduced the primary vector populations in that region, (*Culex tarsalis* Coquillett and *Culex quinquefasciatus* Say) and reduced transmission of WNV (Lothrop et al, 2008). In these cases, the adulticides were applied immediately after the first detection of the virus. The same control of the virus was not seen when the applications were started a month after the first detection, suggesting that regular surveillance and rapid response is a key step in prevention.

Bird feeding *Culex spp.* are the primary vectors of WNV in cases of natural transmission. Three species are of particular importance as primary vectors of WNV in the United States: *Culex pipiens* Linnaeus in the northeast and midwest, *Cx. quinquefasciatus* Say in the southeast, and *Cx. tarsalis* in the west. The onset of human cases of WNV seen to coincide with the host-switching behavior of *Cx. pipiens* in the mid-Atlantic, after the American robin, *Turdus migratorius* Linnaeus, migrates south (Kilpatrick et al, 2006). This was also shown to be true in the case of *Cx. tarsalis* in the Colorado and California. Mosquitoes generally infect humans with the virus in late summer and early fall, after migratory birds have gone south. This is because once the preferred source of food is gone, the questing mosquitoes are left to come down from the tree tops to find another suitable source for a blood meal. Another important factor in the epidemiology of West Nile virus, is that the vectors are turning to human hosts after a season of avian blood meals, during which the virus is amplifying in the host populations (Kilpatrick, 2006).

Zika virus (ZIKAV) is another disease which will be of great concern to public health and mosquito control officials due to its spread into the southern coastal US, and the severity of symptoms resulting from rapid mutations of the virus. Related to dengue and chikungunya, ZIKAV is a *Flavivirus*, and was discovered in 1947 in Uganda (Dick et al., 1951; Kuno et al., 1998). The primary vector of ZIKAV is *Aedes aegypti* Linnaeus, whose northern distribution includes the southwestern regions of Kentucky, but can still be found in low numbers, particularly in the southwest part of the state. This was caused by *Ae. aegypti* having been outcompeted and replaced as the primary anthropophilic mosquito by *Aedes albopictus* Say, after its invasion in the late 1980s (Moore et al. 1988). *Ae. albopictus* is predicted to be an important vector of ZIKAV, though to what extent it will do so in the United States is still uncertain. In some outbreaks, such as in Gabon, 2007, wild specimens of *Ae. albopictus* were collected and shown to be competent drivers of a ZIKAV epidemic in an urban setting (Grard et al. 2014). This was the first time that *Ae. albopictus* has been seen to have such a large role in the spread of ZIKAV. Prior to 2007, only a very limited number of human Zika cases had been reported and Zhu et al. (2016), have attributed the newly emergent epidemic strain of ZIKAV to a single mutation in the non-structural coding region 2B (NS2B), exemplifying the ability of single mutations within the virus to have overwhelming impacts on public health.

As of February 15, 2017, 5,040 total cases of ZIKAV have been reported to the CDC – 220 of which have been the result of local transmission of the disease (Centers for Disease Control and Prevention, 2017). While the locally transmitted cases have been limited to southern Florida (N=214) and southern Texas (N=6), public health professionals around the country are preparing for the introduction of ZIKAV into their states. Models by Monoghan et al. (2016), predict that levels of abundance of *Ae. aegypti* may be moderate to high in the warmer months (July – September) as far north as Kentucky, which may result in elevated risk of *Aedes*-transmitted diseases, specifically ZIKAV. Predictions such as this are important factors in managing surveillance efforts, as observance of *Ae. aegypti* will result in the need for increased control efforts in those areas. Looking specifically at *Ae. albopictus*, Wong et al. (2013) found that in specimens reared from eggs harvested in Singapore, there was a high oral susceptibility of ZIKAV, as well as ZIKAV titres from the midgut and salivary glands. This study was the first to describe transmission of ZIKAV by *Ae. albopictus* as having the ability to transmit ZIKAV, which is of great significance considering that the invasive nature of this species has led it to be spread throughout the US. The likelihood of *Ae. albopictus* to locally transmit ZIKAV in the US is considerable, even in temperate cities, if infected travelers are bitten at a time of high *Ae. albopictus* density, early in the mosquito season, according to models by Manore et al. (2017).

Mosquito Life Histories. One of the most important considerations in providing effective mosquito control is how to best target mosquitoes based on their biological and behavioral traits. Understanding traits such as daytime resting behavior, feeding patterns, and overwintering behavior can allow mosquito control professionals to tailor efforts as effectively as possible. While every species will have unique life history traits, patterns arise at the genus level that can allow one to generalize how best to suppress mosquito populations. The two genera of mosquitoes most common in Kentucky, and which are of greatest public health concern are *Culex* and *Aedes/Ochlerotatus*. The genera *Aedes* and *Ochlerotatus* were made taxonomically distinct in 2000 (Reinert 2000; Reinert et al. 2004), however reanalysis has challenged that change and has again placed *Ochlerotatus* as a subgenus of *Aedes* (Wilkerson et al. 2015). As such, I will refer to *Ochlerotatus* as being grouped within *Aedes*, in following with the Entomological Society of America's recommendation (Reisen 2016). *Anopheles*, *Psorophera,* and *Uranotaenia* are other genera found regularly throughout the state, though their importance to public health is much more limited.

Mosquitoes of the genera, *Culex,* most generally breed in open water, with different species tending to prefer either permanent standing water, or transient water (Service 2008). *Culex restuans* (Theobald) *Culex tarsalis* (Coquiellett), *Culex quinquefasciatus,* and *Culex pipiens* are generally found to breed and develop as larvae in more transient sources of water such as in old fields and drainage ditches (Public-Health Pesticide Applicator Training Manual – Mosquitoes 2017). Eggs of *Culex* mosquitoes are laid upright, in rafts, on the surface of the water. Larvae will develop in water that high in decomposing vegetable matter. The adults of this genus will then spend the majority of their time in sylvan habitats, where they will feed at night on their roosting avian hosts. Kilpatrick et al. (2006) found that upon migration of their preferred hosts, *Culex* spp. will switch hosts, and begin feeding upon mammals.

The most significant *Culex* species to public health in the Unites States are members of the *Cx. pipiens* complex, which includes *Cx. pipiens*, *Cx. quinquefasciatus, Cx. australicus* Dobrotworsky & Drummond, and *Cx. globocoxitus* Dobrotworsky (Knight, 1978), though of the complex, only *Cx. pipiens* and *Cx. quinquefasciatus* are found in the Northern hemisphere. These two species are reported to hybridize, and are thought to do so throughout areas where there is a range overlap, such as in Kentucky (Farajollahi, 2011). *Cx. restuans* is another important vector found throughout much of the United States, and can be very difficult to distinguish from those species of the *Cx. pipiens* complex, though it is not considered a part of that group (Farajollahi, 2011).

Aedes mosquitoes are distributed worldwide, even north into the Arctic where they are able to enter diapause and overwinter as eggs (Service, 2008). This genus tends to breed in habitats with ephemeral water sources such as floodwater areas, marshes, tree holes, and artificial containers. They lay their eggs singly, above the water line and will require submersion in water at least once, but in some cases, several times after desiccation (Service, 2008).

Within the genus *Aedes*, *Ae. albopictus* and *Ae. aegypti* are most relevant to public health, because of the number and severity of pathogens that they vector, including dengue, and Chikungunya. *Ae. aegypti* is the more serious of the two species, because it is also the primary vector of yellow fever. *Ae. aegypti* was introduced into the United States from Africa on ships during the transatlantic slave trade as early as the 1640s (Powell and Tabachnick, 2013) and was at one time the primary nuisance mosquito throughout much of the United States, responsible for many yellow fever outbreaks including the last one, in New Orleans, Louisiana, 1905. A widespread eradication program led by Pan American Health Organization between 1948 and 1962 led to the elimination of *Ae. aegypti* in all but the southeastern United States and much of the Western Hemisphere, until efforts were halted in the 1970s and it reemerged (Powell and Tabachnick, 2013). Another invasive species, *Ae. albopictus*, was introduced in Houston, Texas by way of used tire shipments from Asia (Sprenger and Wuithiranyagool, 1986). The mosquito has since spread throughout most of the continental United States and Hawaii. It is much better adapted to low temperatures than *Ae. aegypti* and will outcompete it at the larval stage in temperate climates (Juliano and Lounibos, 2005). Both *Ae. albopictus* and *Ae. aegypti* are highly anthropophilic container breeding species, however, *Ae. albopictus* is not as dependent on humans as *Ae. aegypti*, as it tends to be a forest edge dwelling species (Moore et al., 1988). *Aedes vexans* Meigen is also important for its ability to act as a bridge vector of WNV (Molaei and Andreadis, 2006) and as a vector of dog heartworm (*D. immitis*) (Ledesma and Harrington, 2011).

Mosquito Control. In the past, mosquito control relied heavily on the widespread application of dicholorodiphenyltrichloroethane (DDT), the great success of which spurred the development of other organochloride insecticides throughout the 1940s and 1950s (Thacker, 2002). DDT is a very persistent, fat-soluble, neurotoxin which results in its biomagnification in ecosystems, becoming toxic to organisms at higher trophic levels. It was also implicated in the decline of predatory birds, because it thinned eggshells by affecting calcium metabolism (Thacker, 2002). For these reasons, it has been banned in the United States, though it is still used in developing countries to combat mosquitoes (Thacker, 2002). Post-WWII, synthetic pyrethroids were developed from pyrethrum, an extract of chrysanthemums and are now some of the most commonly used insecticides in mosquito control programs.

The application of residual barrier sprays, has become a popular option among pest management professionals, as many studies have validated their effect at suppressing mosquito populations on small spatial scales (Madden et al., 1947; Trout et al., 2007; Muzari et al., 2014; Li et al., 2014). In a typical barrier spray, a pest management professional will apply a residual insecticidal treatment around the perimeter vegetation of a backyard, as well as treating under other suspected daytime resting places, such as decks and porches. Such applications are effective at suppressing local populations as the majority of mosquitoes resting in the vegetation during the treatment will be killed and others from outside the perimeters that have contact with the treated vegetation while questing for a blood meal are also killed. The most recent of these studies have used synthetic pyrethroids, particularly lambda-cyhalothrin (Trout et al., 2007; Li et al., 2014; Muzari et al., 2014). The typical barrier treatment has been found to be most effective against *Aedes spp*. as opposed to *Culex spp.,* which tend to rest too high up in the foliage and don't come into contact with the residual insecticide (Trout et al., 2007). Cilek and Hallmon (2008) found that in comparing lambda-cyhalothrin to beta-cyfluthrin and tau-fluvalinate in field cage trials, the reduction in abundance of both *Cx. quinquefasciatus* and *Ae. albopictus*, was best with lambda-cyhalothrin, indicating that it is likely a behavioral issue resulting in lack of control of *Culex spp*. Given the importance of *Culex spp.* as vectors of disease and the difficulty of suppressing species of this genus, the development of a more novel approach to mosquito control is necessary.

Unfortunately, resistance to synthetic pyrethroids has become a serious problem in vector control throughout the world. Resistance to lambda-cyhalothrin, specifically, has been shown in *Anopheles gambiae* Giles (N'Guessan 2007), *Cx. pipiens pipiens* and *Cx. pipiens molestus* in Morocco (Bkhache 2016), and *Ae. aegypti* in northern Mexico (Chino-Cantor 2014). As a response to the concerns of environmental toxicity of insecticides and their lessened effectiveness through the evolution of resistance, the application of integrated pest management (IPM) strategies to mosquito control were suggested beginning in the 1970s (Axtell 1979). The components of IPM include cultural/physical, biological, and chemical. An example of such a strategy on the scale of a suburban backyard would be to drain standing water from containers, gutters, and flooded areas of the lawn, applying a residual barrier spray, and adding a larvicide to more permanent bodies of water.

In an attempt to increase control of mosquitoes, novel formulations and applications of insecticide active ingredients are being explored. One such chemical Pyriproxyfen is an insect growth regulator (IGR) which acts as a juvenile hormone mimic, resulting in the death of mosquito pupae, through the disruption of normal development of the immature stage of the insect (El-Shazly et al., 2002). This IGR has been found to be an effective larvicide of *Ae. albopictus* (Ali et al., 1995) and *Cx. pipiens* (El-Shazly et al. 2002) in very small amounts (LC_{50}, 0.00011ppm at 20°C), at a wide range of temperatures, making it a good fit for addition to outdoor mosquito control efforts. In addition to acting as a larvicide, pyriproxyfen will also reduce fecundity of females exposed to it before taking blood meals (Itoh 1994) and can be disseminated to larval habitats by gravid females who have come into contact with it through exposure to pyriproxyfen treated surfaces (Itoh 1994; Chism and Apperson 2003). These studies have used a pyriproxyfen formulated in granules, which are physically picked up on the tarsi of females when they land on the treated surfaces. They then carry those granules to oviposition sites where they are transferred and dissolved into the water when eggs are being laid. Field studies testing the hypothesis of autodissemination of pyriproxyfen from adult resting sites to larval habitat have supported the previously noted laboratory trials. Devine et al. (2009) found 95-100% mortality of *Ae. aegypti* larvae developing in sentinel oviposition cups placed near pyriproxyfen dissemination stations consisting of a plastic cup lined with moistened cloth dusted with powdered pyriproxyfen. At a neighborhood scale, application of pyriproxyfen to point-source (applied to tire piles with a backpack sprayer) and area-wide (applied with ULV truck-mounted sprayer) resulted in dissemination of pyriproxyfen to sentinel cups in both treatments, however only the point-source treatments reduced abundance of mosquitoes as determined by trap counts (Suman et al., 2014).

Currently, the lack of control for *Culex spp.* and the widespread abundance of invasive *Aedes spp*. are of great concern given their threat as vectors in the Unites States. The threat of mosquito-borne disease has only increased in recent years and will likely continue to do so as globalization aids in the spread of vectors and pathogens to naïve populations. As more effective mosquito control measures are sought, novel uses of pyriproxyfen, such as its use in barrier treatments are of great interest to PMPs. It is this approach that will be the topic of this research, as we seek to gain additional tools in protecting the public from vector-borne disease.

Objectives

(1) To evaluate if the addition of pyriproxyfen to the synthetic pyrethroid, lambda-cyhalothrin, will lengthen the suppression of mosquitoes when applied with a backpack sprayer to suburban backyards.

(2) To determine the residual duration of pyriproxyfen on treated containers of two different materials exposed to a backyard environment.

(3) To evaluate suppression of mosquitoes achieved through the application of lambda-cyhalothrin with a truck-mounted mist blower along tree lines, both with pyriproxyfen and alone.

CHAPTER I

Addition of Pyriproxyfen to a Tank Mix with the Synthetic Pyrethroid, Lambda-cyhalothrin to Lengthen the Effective Duration of Mosquito Control when Applied with a Backpack Sprayer to Suburban Backyards

Introduction

The first residual sprays were used for mosquito suppression indoors, in primarily tropical and sub-tropical areas where large numbers of the population are at risk of contracting mosquito borne diseases such as dengue and malaria (Ansari et al., 1986). The earliest sprays used DDT, but as that fell out of use with mosquito control professionals, experimentation began with other insecticides and methods of control (Pant et al., 1974; Lofgren, 1974). More reliance was later placed on an integrated pest management approach and widespread control measures such as release of sterile males and aerial applications of ultra-low volume insecticide.

The West Nile Virus (WNV) outbreaks that occurred across the US in the early 2000s, saw numbers of neuroinvasive disease cases reaching 2,946 in 2002 and 2,866 in 2003 (CDC 2016). The disease resurged in 2012 with 2,873 cases reported nationally. Ornithophilic *Culex spp*. of the *Cx. pipiens* complex are the most common vectors of WNV, though other species and genera are capable of transmitting the virus experimentally. After WNV became a publicly known health concern, many pest control companies began offering backyard mosquito control services as a way to lower the abundance of mosquitoes on individual properties (Blake 2013). These services rely on the use of residual synthetic pyrethroids, such as lambda-cyhalothrin, bifenthrin, and cyfluthrin.

Previous studies from the Public Health Entomology Laboratory at University of Kentucky were some of the first to test the efficacy of the outdoor perimeter sprays (Trout et al. 2007). These sprays rely on the application of residual insecticides to perimeter vegetation and other daytime resting places of mosquitoes, so that mosquitoes are killed when they seek harborage when they are not active. Other studies have confirmed the long residual duration of lambda-cyhalothrin and successful use in barrier spray applications in many different geographic locations with varying climates (Cilek et al. 2008; Britch et al. 2009; Li et al. 2014; Muzari et al. 2014). These applications are not always enough to satisfactorily suppress mosquito populations with a single application, or perhaps in areas where pyrethroid resistance has become a hurdle for mosquito control professionals. As a result, the addition of multiple-active ingredients to help boost the lethality and duration of conventional sprays are being examined.

One such active ingredient is pyriproxyfen, an insect growth regulator (IGR). This chemical is known to cause high mortality of immature mosquitoes in very minute quantities (Itoh et al. 1994) and to have effects on the fecundity and fertility of females in sublethal doses (Hamburguer et al. 2014). Laboratory studies have confirmed the ability of adult mosquitoes to disseminate pyriproxyfen (Itoh et al. 1994; Chism and Apperson 2003; Wang et al. 2013; Devine 2016) and now field trials are investigating the ability of applying this concept to mosquito control efforts in outdoor habitats (Devine et al. 2009 and Suman et al. 2014). This study will aim to contribute to these field-based applications through the addition of pyriproxyfen to a backpack mist blower for the use in backyard perimeter sprays. Pyriproxyfen will be combined with the synthetic pyrethroid, lambda-cyhalothrin, under the hypothesis that female mosquitoes will be able to disseminate it to their breeding sites during oviposition. In addition, it is hypothesized that control will be enhanced through the lowered fecundity of females when they are exposed to pyriproxyfen treated foliage.

I will also test the residual efficacy of pyriproxyfen when applied to two different materials of containers, often found as breeding habitats for container breeding species such as *Aedes albopictus* Skuse and *Aedes aegypti* Linnaeus. Bioassays have been conducted using laboratory treated materials against stored product pests, but a review of the literature as performed by Arthur et al. (2009) did not return studies on the residual efficacy of containers treated and exposed to field conditions. Suman et al. (2013), found that different substrates will impact the efficacy of pyriproxyfen and hypothesized that this was the result of absorption of the product into the materials. They also found a relationship between pH levels, which changed as a result of the materials and the findings suggest that low and high pH levels have an additive effect on mortality, when combined with pyriproxyfen. Their work took place in laboratory trials and did not account for variability of field conditions. I will test the residual efficacy of pyriproxyfen when applied to containers and exposed to field conditions with bioassays hypothesizing that porous containers may allow for absorption into the substrate, lowering the exposure of larvae to the chemical. This will also allow for the collection of leave material and other organic debris, into which pyriproxyfen may be absorbed.

Methods and Materials

A total of 30 homeowner volunteers were recruited to allow use of their property during 2015. Recruitment took place in late May/early June with the use of printed door hangers in neighborhoods of similar age. Once a response was received from a homeowner, the properties were inspected to insure adequate perimeter vegetation. Examples of vegetation commonly found in the backyards includes flowering annuals and perennials (<1.0m), hedges and shrubs (~0.5-2.0m), saplings and small trees (~2.5 – 10.0m), and mature trees (~10.0 – 30.0m). Almost every yard had at least one Japanese honeysuckle, *Lonicera japonica* Thunberg, so it's use in bioassays and trap placement when possible, allowed for lowered variability between plots. While little is known about mosquito preference for the vegetation which serves as a daytime resting place, Japanese honeysuckle provides dense cover and a nectar source when flowering (which would be prior to application of a perimeter treatment) and so it is thought that it would be preferable for use as a resting place. In addition, it is native to Japan, as is the biotype of *Ae. albopictus*, the primary nuisance mosquito found in Kentucky.

Houses with no perimeter vegetation do not allow for application of treatments and were excluded from the study. Pre-treatment sampling began

June 16, 2015 and continued through July 1, however as a result of spatial overlap with another study which required alternate sites to be chosen, only 10 homes were able to be sampled from, all of which were consequently assigned to insecticidal treatments. As a result, pretreatment sampling was not included in analysis.

Three treatments were used and replicated 10 times: lambda-cyhalothrin (Demand® CS, 6.25ml/l, Syngenta Crop Protection, Greensboro, NC), lambda-cyhalothrin (6.25 ml/l) + pyriproxyfen (Archer® 7.81ml/l, Syngenta Crop Protection, Greensboro, NC), water control applied to grass only. The control treatments were applied only to grass, because previous work performed by the lab suggested that applying the water control to perimeter vegetation would alter homeowner satisfaction surveys by displacing mosquitoes the first night of treatment. It was previously observed however, that mosquito levels in the controls applied to perimeters would return to those seen pretreatment within a week's time. Treatments were applied July 6, 2015 by a certified pest control technician from All-right Pest Control Inc., Lexington KY. The treatments were applied when the vegetation was dry from any dew or rain and when there was little to no wind. Application was performed using a Stihl backpack mist blower (Model SR-420, Stihl Corp, Virginia Beach, VA.). The sprays were applied with in an up-down motion, until vegetation was misted to the point just before run-off would occur. The lower branches of some mature trees were treated when chemical trespass (the spraying of insecticide treatments onto neighboring properties) was not an issue and these branches were not generally more than 3m high. Care was taken to treat the inner branches of more dense foliage, by inserting the tip of the mist blower into the vegetation. To reach non-vegetative resting places, structures such as the undersides of decks, sheds, and woodpiles were sprayed.

Mosquito Monitoring. Beginning the first week post-treatment, all properties were sampled for mosquitoes on weeknights from July 14 - Sept. 9, 2015. Five different sampling methods were used at each property: (1) Centers for Disease Control (CDC) miniature light traps (Model 512, John W. Hock, Gainseville, FL), (2) CDC gravid traps (Model 1712, John W. Hock, Gainesville, FL), (3) Ovitraps made from black plastic, 12cm diameter, (4) Human Landing Rates were taken from 1600h – 1900h and sampled the actual number of mosquitoes landing/biting on an unprotected person for 5 minutes. (5) Finally, leaf samples were taken for use in a bioassay, returned to the laboratory, placed in a vial with *Aedes albopictus* Skuse adults and mortality assessed after 24h and 48h. After the mosquitoes were collected from the field, they were placed in a cooler and taken back to the lab, where they were frozen, and identified to species, and counted.

CDC miniature light traps were placed in the back corners of properties between 1600 and 1000, in front of a patch of foliage, if available. They were baited with a light (Type: CM-47, 150 mAmp/hr) and approximately 2.3 kg of pelleted dry ice. The dry ice was packed into 1.89 L coolers (“Contour™ 0.5” Gallon, Igloo Products Corps., Houston, TX). CO_2 was able to escape through the opened top spout, two holes drilled into the sides, and one hole drilled in the bottom into which a clear Tyson tube (0.5” OD x 3/8” ID Vinyl Tubing, Model 089) cut to 0.6 m was inserted to deliver the flow of CO_2 directly towards the top of the trap. The CDC light traps were used primarily to attract female mosquitoes questing for a blood meal and will also catch a higher percentage of *Aedes/Ochlerotatus spp.* than *Culex spp.* or others (Trout et al., 2007).

Gravid traps were set out directly under the CDC miniature light traps between 1600 and 1000 and were baited with 4 l of grass-infused water. These traps are designed to catch ovipositing females and because of the water infusion will generally attract a higher percentage of *Culex spp.* The grass-infused water was made by mixing 19 l of water with 100 g of cat chow (Friskies®, Nestlé Purina Petcare, St. Louis, MO, USA), approximately 0.5 g of fescue grass, and 0.5 ml TopFin® Tap Water Aquarium Dechlorinator Water Conditioner (PetSmart Inc., Phoenix, AZ).

Human landing rate counts were conducted weekly at each property from 1600 to 1800. The laboratory director performed these counts exposing the skin on the thigh and shin for 5 minutes in the same area of the lawn where the traps were located. Mosquitoes were counted when they landed on the skin, regardless of whether or not they bit the one performing the counts.

Ovitraps were black plastic cups (500 ml) lined with egg paper (76 lb. seed germination paper, Anchor Paper Co., Minneapolis, MN) and filled with the same grass-infused water used in the gravid traps. At each property, one cup was hung from a branch at 1.5 m. Collections from the ovitraps were taken weekly. The larvae were taken from the traps back to the lab, where they were stored in the refrigerator until killed and counted.

Laboratory Bioassays. Adult bioassays were performed to test the residual toxicity of the insecticide on leaves collected from the treated vegetation. Weekly, at each site, one leaf was collected from outer deciduous vegetation at approximately 1.5 m height. Japanese honeysuckle was used at 28 of the sites for the bioassays and plants similar in leaf size and texture were chosen at the remaining two sites that did not have Japanese honeysuckle on the property. This done to limit variability in leaf surface texture and because it was suspected to be a likely harborage for day resting mosquitoes. The collected leaves were picked with gloved hands, placed in individual bags, and refrigerated until the bioassays were setup within 24 h of collection. The bioassays used 7 dram plastic vials (Acorn Naturalists, Tustin, CA) that had the bottoms removed with a drill press, over which fine mesh was hot-glued to allow for ventilation within the vial. The leaves were taken out of refrigeration, handled with gloves changed between treatments, and placed onto the vials abaxial side down. Each vial contained five laboratory reared *Ae. albopictus* and were stored in a growth chamber set at 27°C and 75% R.H. for 48 h. At 24 h intervals, the bioassays were evaluated for mortality, which was determined when mosquitoes did not respond with movement to stimulation – mortibound individuals were assessed as alive.

Backyard Container Bioassays. The container trial was set up in a suburban backyard from August 8, 2016 to September 9, 2016. Ten each of two kinds of containers were used; 900 ml metal paint cans and 900 ml plastic paint mixing cups. Five of both material were then dipped in a mid-label rate solution of pyriproxyfen (Archer® 7.81ml/l, Syngenta Crop Protection, Greensboro, NC). The containers were randomly placed upon a 183x76 cm folding table (Lifetime Products®, Model #80295, Clearfield, UT) and filled with 500 ml of distilled water. The water level was monitored and maintained at 500 ml throughout the study. Water samples were collected weekly and used in larval bioassays, which followed the same procedure as was used with water samples from the field locations.

Homeowner Satisfaction Surveys. Homeowners were surveyed just before treatment, 4 weeks post-treatment, and 8 weeks post-treatment. The surveys assessed the general attitude of the homeowners toward mosquitoes in their yards, their satisfaction with the treatment, and other relevant information. In addition, homeowners were also given a weekly log in the form of refrigerator magnets, which were to be completed weekly, for 8 weeks. If the homeowners were away for that week, they were instructed to leave the survey blank. The surveys asked the homeowners to select the rank they felt best represented the perceived pressure of mosquitoes in their yards. The survey ranks correlated to homeowner behavior in response to mosquito pressure as:

<u>Rank</u> <u>Behavior</u>

1. We did not notice any mosquitoes
2. Not enough to use repellents or to avoid outdoors.
3. At least some of us were bothered by mosquitoes to use protective measures, (e.g. repellents) or avoid being outdoors.
4. Mosquitoes were very noticeable and were a definite annoyance most of the week.
5. Mosquitoes were very bad this week.

The logs were collected after completion of the study.

The responses to the surveys can be found in Appendix I.

Statistical Analysis. All statistical analyses were performed using JMP 12.1 (SAS Institute 2015). Mosquito bioassays were adjusted using the Schneider - Orelli correction (Puntener 1981). Schneider - Orelli correction:

$$\text{Corrected } \% = \left(\frac{\%\text{ Mortality treated} - \%\text{ Mortality control}}{100 - \%\text{ Mortality Control}} \right) * 100$$

The bioassay percent reductions were untransformed and analyzed with Tukey's test. Larval data was non-normal and therefore analyzed using the Kruskal-Wallis (Kruskal 1952) test, and the Steel-Dwass method for nonparametric multiple comparison was used. Backyard bioassays were analyzed using a chi-square approximation. The number of mosquitoes trapped at each location, were normalized using the square-root transformation and analyzed using ANOVA and Tukey-Kramer comparison of means. The model was constructed using both the week and treatment variables as combined effects. Abbott's formula (Abbott 1925) was used to calculate the percent reduction in mosquito populations in each yard. Abbott's formula:

$$\text{Corrected } \% = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) * 100$$

Where: n = insect sample, T = treatment, and Co = control

Results and Discussion

Mosquito Monitoring. Over the course of the 9 week study, 9,750 mosquitoes were collected from the CDC traps and the gravid traps combined (Table 1.1). The gravid traps captured 55% and the CDC traps 45%, of all mosquitoes caught. There were 11% of specimens, a rather high percentage, which were too badly damaged to identify. This was a result of the trap types used requiring insects to pass through the fans before falling into the nets, as well as the traps being caught in rain before collection. Males were not included in further analyses, but amounted to 4.7% of the total collection. *Aedes/Ochlerotatus spp.* made up nearly 47% of all collected mosquitoes and nearly 28% were *Culex spp.* Other genera collected include: *Anopheles spp.*, *Psorophora spp*, *Uranotaenia sappharina*, and *Orthopodomyia spp*. The three most commonly caught species were *Cx. pipiens* (29%), *Ae. albopictus* (23%), and *Aedes vexans* Meigen (18%). CDC traps caught 4,408 mosquitoes throughout the season. *Aedes spp*. accounted for 68% of those trapped, 12% were *Culex spp.*, 8% males, 6% *Anopholes spp.*, and other genera making up the remaining 6%. Gravid traps caught 5,342. The breakdown of genera for these traps is as follows: 28% were *Aedes spp.,* 52% *Culex spp*., 17% unidentifiable, and the remaining 3% were other genera. Of all the *Aedes spp.* collected, 67% were from CDC traps and of *Culex spp.,* 85% were collected from gravid traps.

The species captured throughout the season were comparable in number and proportion to surveys collected by Trout et al. (2007), with a few notable exceptions. This includes the presence of *Culex nigripalpus* Theobald, an important vector of St. Louis encephalitis, and one whose abundance has been shown to precede outbreaks of the disease (Day 1993; Day 2001). The other two species not caught in 2007, were *Ochlerotatus japonicus* Theobald, a competent vector of WNV (Sardelis et al. 2001) and *Uranotaenia sappharina* Osten Sacken.

Human Landing Rates. Human landing rate counts showed a significant treatment effect (F = 56.06; df = 2, 223; P = 0.0001), but not week effect (F = 0.5246; df = 2, 223; P = 0.8154). Treatment by week analysis of least squared means showed significant reduction in landing rates through week 7 for the lambda-cyhalothrin treated yards (T = -5.04; df = 2,27; P = 0.0116), and through week 8 for the lambda-cyhalothrin + pyriproxyfen treated yards (T = -2.52; df = 2,29; P = 0.0461) (Figure 1.1). No significant difference was detected between the lambda-cyhalothrin and lambda-cyhalothrin + pyriproxyfen treatments. The lambda-cyhalothrin treated yards saw a reduction in landing rate counts by a cumulative mean of 80% after 4 weeks post-treatment, 71% for 6 weeks post-treatment, and 64% for 8 weeks post-treatment. The addition of pyriproxyfen to the tank mix resulted in cumulative mean reductions of 85% for 4 weeks post-treatment, 76% for 6 weeks post-treatment, and 71% for 8 weeks post-treatment.

Bioassays. The bioassays showed significant treatment effects (F = 45.97; df = 2,178; P = <0.0001) and treatment week interactions (F = 13.5003; df = 9,178; P = <0.0001) at the 24 hour interval (Figure 1.2) and these became even stronger at the 48 hour interval (Figure 1.3) (F = 82.66; df = 2,178; P = <0.0001 and F = 19.5386; df = 9,178; P = <0.0001, respectively). The Tukey-Kramer analysis showed that at 24 hours, there was significant mortality from leaves of the lambda-cyhalothrin treatment (T = 8.35; df = 169; P = <0.0001) and from the lambda-cyhalothrin + pyriproxyfen treatment (T = 9.03; df = 169; P = <0.0001) compared to the control, but there was no significant difference between the insecticidal treatments (T = -0.69; df = 169; P = 0.7695). The same analysis run for the mortality assessments taken at 48 hours showed stronger significance for mortality when exposed to lambda-cyhalothrin (T = 11.01; df = 169; P = <0.0001) and lambda-cyhalothrin + pyriproxyfen treatments (T = 11.04; df = 169; P = <0.0001), but again no significant difference between them (T = -0.44; df = 169; P = 0.8995).

Ovitraps. The difference in mean larvae caught in the cups showed a significant week effect (X^2= 40.11; df = 7; P = <0.0001) as well as a significant treatment effect (X^2 = 12.34; df = 2; P = 0.0021) (Fig 1.4). The Steel-Dwass method of analysis revealed a significant difference between the lambda-cyhalothrin + pyriproxyfen treatment and the control ($Z = 3.4$, q* = 2.34; P = 0.0020).

Trap Analysis. Over the course of the summer, the mean number of mosquitoes caught on each property/week was 26.3 ± 2.58 from yards in the lambda-cyhalothrin treatment, 31.7 ± 3.15 from yards in the lambda-cyhalothrin + pyriproxyfen treatment, and 45.6 ± 4.16 from those in the control. The overall reduction in mosquitoes caught from properties treated with lambda-cyhalothrin alone was 40% at 4, 6, and 9 weeks. At lambda-cyhalothrin + pyriproxyfen treated homes, the overall reduction was 32% at 4 weeks, 34% at 6 weeks, and 35% at 9 weeks. The combined CDC and gravid trap results showed a significant treatment week interaction effect (F = 38.23; df = 10, 258; P < 0.0001), as well as significant week (F = 42.43; df = 8; P < 0.0001) and treatment effects (F = 22.99; df = 2; P < 0.0001). The difference of least squared means showed lambda-cyhalothrin (T = -6.47; df = 258; P < 0.0001) and lambda-cyhalothrin + pyriproxyfen (T = -4.55; df = 258; P < 0.0001) both differing significantly from the control, but not from one another (T = -1.93; df = 258; P = 0.1321). This difference from the control was seen as late as week 8 post-treatment on the properties treated with lambda-cyhalothrin only (T = -2.62; df = 27; P = 0.0366), however this was not a consistent trend throughout the weeks post-treatment (Figure 1.5).

The results of treatments differed when trap types were analyzed separately, with CDC traps showing a greater reduction of mosquitoes than was seen by the results of the gravid traps. CDC traps caught a mean of 10 $\pm$ 0.9 mosquitoes from the lambda-cyhalothrin only treated properties, 11 $\pm$ 1.2 from those yards in which the treatment added pyriproxyfen, and 24 $\pm$ 2.5 in the yards serving as controls. In lambda-cyhalothrin treated yards, the reduction of mosquitoes was 54% 4 weeks post-treatment, 50% 6 weeks post-treatment, and 44% 9 weeks post-treatment. A comparable level of reduction was seen in the yards assigned to the lambda-cyhalothrin + pyriproxyfen treatment, being 51%, 48%, and 45%, respectively. The statistical analysis of CDC traps only, revealed significant treatment (F = 24.61; df = 2; P < 0.0001), week (F = 13.08; df = 8; P < 0.0001), and treatment week interaction (F = 15.44; df = 10, 268; P < 0.0001) effects. The Tukey-Kramer analysis suggested a significant difference between both treatments, lambda-cyhalothrin (T = -6.21; df = 258; P < 0.0001) and lambda-cyhalothrin + pyriproxyfen (T = -5.94; df = 258; P < 0.0001), and the control, but again, not between each other (P > 0.05) (Figure 1.6).

The gravid traps caught an average of 16 ± 2 mosquitoes in the lambda-cyhalothrin only treated yards, 21 ± 2.7 in the lambda-cyhalothrin + pyriproxyfen treated yards, and 22 ± 2.6 in the controls. The overall reduction of mosquitoes on those properties treated with lambda-cyhalothrin was 30% 4 and 6 weeks post-treatment, and 32% after 9 weeks. In the yards in which pyriproxyfen was added, the reduction was 25%, 26%, and 30%, 4,6,and 9 weeks post-treatment. There was a significant week (F = 50.75; df = 8; P = 0.0011) and treatment week interaction (F = 41.44; df = 10; P < 0.0001) effect, but not a significant treatment effect (Figure 1.7)

There were also differences in the levels of control based on the genera of mosquitoes. In yards treated with lambda-cyhalothrin, *Aedes* mosquitoes were reduced by 59% after 4 weeks, 54% after 6 weeks, and 46% after 9 weeks post-treatment (Figure 1.8). Lambda-cyhalothrin + pyriproxyfen treated properties saw a reduction of 51%, 43%, and 42% after the same number of weeks post-treatment. This is in contrast to *Culex spp.* in which the reduction was 23%, 22%, and 26%, 4, 6, and 9 weeks post-treatment in the lambda-cyhalothrin only yards and 20%, 17%, and 19% when pyriproxyfen was added (Figure 1.9). There were significant week (F = 11.0; df = 8; P < 0.0001), treatment (F = 40.41, df = 2; P < 0.0001), and treatment week interaction (F = 16.94; df = 10, 268; P < 0.0001) effects. Both treatments reached a significant difference in mosquito suppression from the control – lambda-cyhalothrin (T = -8.10; df = 258; P < 0.0001), lambda-cyhalothrin + pyriproxyfen (T = -7.44; df = 258; P < 0.0001) – though again, no difference between the treatments. The *Culex* mosquitoes saw a reduced treatment effect (F = 4.32; df = 2; P = 0.0142), and difference in treatments between lambda-cyhalothrin + pyriproxyfen and the control (T = 2.69; df = 258; P = 0.0209) and lambda-cyhalothrin + pyriproxyfen and the lambda-cyhalothrin only treatment (T = -2.38; df = 258; P = 0.0475).

Backyard Container Bioassays. The results of the bioassay (Fig. 1.10) showed that there was a significant treatment effect (X^2 = 35.4; df = 1; P < 0.0001) and week effect (X^2 = 17.2; df = 3; P = 0.0006). The material had no significant effect however. This result is similar to those of Arthur et al. (2009), who tested the residual efficacy of pyriproxyfen on dry surfaces of wood, concrete, and metal against stored product pests in a laboratory. The exposure to field conditions and submergence in water did not lower pyriproxyfen's efficacy against mosquito larvae.

Homeowner Satisfaction Surveys. The results of the "Pretreatment" Homeowner Satisfaction surveys showed that 67% of respondents either "Agreed" or "Strongly Agreed" that mosquitoes limited their outdoor experience and 77% "Agreed" or "Strongly Agreed" that they worried about the health threat of mosquitoes. When asked if they believed that Lexington had a mosquito problem, 60% responded "Yes", while 37% were "Unsure", and only 3% gave a definitive "No". Homeowners said on average, that 4.4 mosquito bites indicates a mosquito problem. In regards to their mosquito control expenses, 80% spend $0 throughout the year. The "Post-Treatment" surveys revealed that a total of 57% of Homeowners felt that the treatment they received adequately controlled the mosquito populations in both July and August – 100% of those from the lambda-cyhalothrin treatment, 50% from lambda-cyhalothrin + pyriproxyfen treatment, and 40% from the control. 64% said that the treatment they received allowed them to stay outside longer, 67% of those from both insecticide treatments, and 40% of the control. Of those who said the treatments allowed them to stay outside longer, 64% chose 2-4 hours longer, of almost the same percentage among treatments – 67% lambda-cyhalothrin, 60% lambda-cyhalothrin + pyriproxyfen, and 67% control. All of those who responded agreed that the treatments they received reduced bites. When asked about negative side-effects of the treatment, 85% said that there were none, with the 15% who chose "Other_______", leaving the field blank. Overall, 85% of the homeowners said that the mosquito situation in their yard was "Much better" – including 100% of those receiving the lambda-cyhalothrin treatment and controls, while only 60% of those who received the added pyriproxyfen. It is notable that although they received no chemical treatment, there was a considerable placebo affect seen with the responses from the homeowners assigned to the control group.

Conclusions

Both lambda-cyhalothrin and lambda-cyhalothrin + pyriproxyfen significantly suppressed mosquito populations in suburban backyards of Lexington, KY. There was no difference however between the two insecticide treatments. The addition of pyriproxyfen did not appear to detectably increase the effective duration of backyard perimeter sprays targeting peridomestic mosquitoes to any statistically significant degree. The length of mosquito suppression with the use of lambda-cyhalothrin over this field season was comparable to those seen in similar studies of this kind (Trout et al., 2007; Cilek et al., 2008; Britch et al., 2009), though the percent reduction was slightly lower than what would have been expected, as the aforementioned studies saw levels of reduction upwards of 80-90% over similar time periods.

The difference in the results based on trap type is likely do to the differences in behavior between mosquitoes questing for a blood meal and those in search of suitable oviposition habitat. The mosquito genera that are generally attracted to the different trap types will also be an important factor in measuring the levels of control seen in these types of studies, such as the tendency for *Culex* mosquitoes to be caught in very high numbers by Gravid traps, particularly when baited with grass water to specifically lure them to the trap. This is especially true in studies like this, where the majority of the specimens captured were done so with gravid traps, increasing the likelihood that they be *Culex spp.* or gravid females. As both of these are less likely to exhibit behavior that would encourage their contact with the active ingredients, they will not show the impact that can be had by barrier sprays on mosquitoes most likely to bite and infect a human with a mosquito-borne disease.

Recent studies have supported the use of pyriproxyfen in field applications of insecticide for its ability to cause high pupal mortality even in minute quantities, to be autodisseminated by adult mosquitoes to breeding sites and for the reduced fecundity of females who come into contact with it (Itoh, 1994; Devine et al., 2009; Wang et al., 2013; Suman et al., 2014). However, the majority of these studies have included a point-source method of dissemination and recent work by Suman et al. (2014) suggests that area-wide applications of pyriproxyfen using aqueous formulations are not well-suited to use in barrier sprays as mosquitoes are not able to pick up and disseminate an adequate amount of the active ingredient to breeding sites. Although this study employed the use of a mist-sprayer with the goal of providing a higher volume of spray than a ULV application would, it appears to be the case that in the small spatial scale of a backyard, any increased suppression of mosquitoes is not being detected or is being quickly replaced by mosquitoes who are able to fly over or through the treated vegetation from untreated neighboring yards. Further work would benefit from testing the use of pyriproxyfen in barrier treatments as a means of providing auto-dissemination of the product to breeding sites and the lowered fecundity of female mosquitoes in larger-scale treatment areas, such city parks and cemeteries.

Table 1.1, Total mosquitoes collected in 30 suburban backyards (6 July – 9 September 2015) from Lexington, KY. These include mosquitoes caught from both insecticide treated and control yards.

Species	CDC Trap	Gravid Trap	Number	% Total
Cx pipiens	320	2496	2816	28.88
Ae albopictus	923	1355	2278	23.36
Ae vexans	1645	65	1710	17.54
Oc trivitattus	359	32	391	4.01
Cx spp.[1]	9	216	225	2.31
An punctipennis	191	22	213	2.18
Cx erraticus	140	18	158	1.62
Ae spp.	33	17	50	0.51
An quadrimaculatus	39	18	57	0.58
Oc japonicus	41	19	60	0.62
An perplexans	50	2	52	0.53
Cx nigripalpus	17	12	29	0.30
Oc triceriatus	22	6	28	0.29
Cx salinarius	10	26	36	0.37
Ur sapphirina	25	0	25	0.26
Oc hendersoni	11	6	17	0.17
Ps horrida	13	3	16	0.16
Ps spp.	6	4	10	0.10
Cx restuans	8	1	9	0.09
Cx territans	4	2	6	0.06
Ps ferox	5	0	5	0.05
Ps columbeae	2	0	2	0.02
An spp.	1	1	2	0.02
An barberi	0	1	1	0.01
An crucians	1	0	1	0.01

Table 1.1, Continued

Species	CDC Trap	Gravid Trap	Number	% Total
Oc cinerius	1	0	1	0.01
Or alba	1	0	1	0.01
Or signifera	1	0	1	0.01
Or spp.	1	0	1	0.01
Ps cyanescens	1	0	1	0.01
Unknown	188	903	1091	11.19
Males	340	117	457	4.69
Total	4408	5342	9750	100

[1] Spp. indicates specimens collected were too badly damaged to identify to species.

Figure 1.1, Mean (± SEM) mosquitoes observed landing on human subject every five minutes per week in 30 suburban backyards (6 July – 9 September 2015) from Lexington, KY between 1600 and 1800 hours.

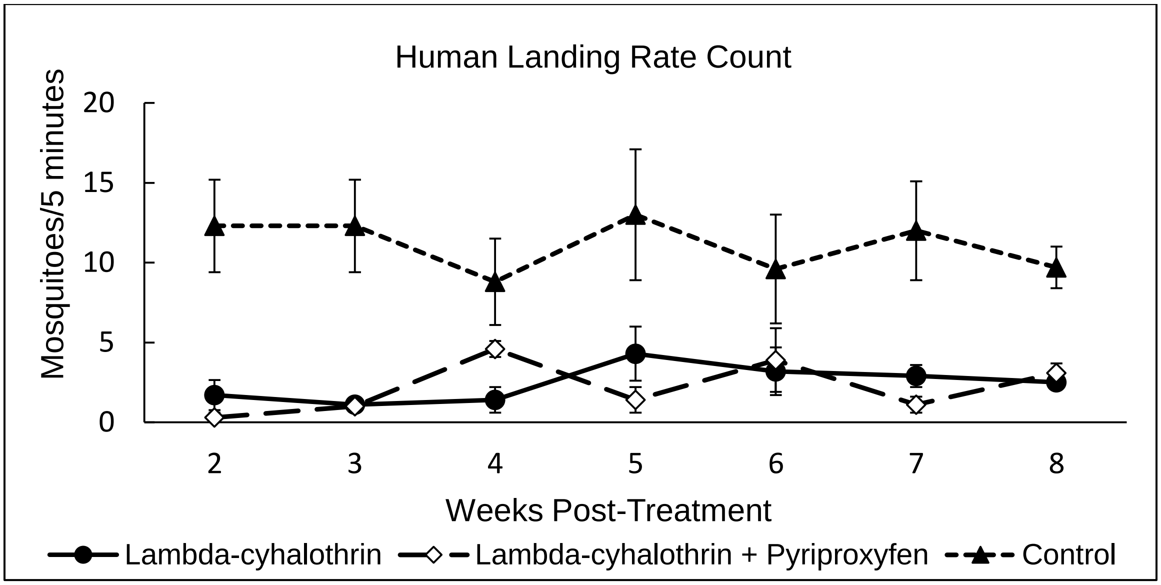


Fig 1.2, Mean (± SEM) corrected percent mortality of lab-reared *Ae. albopictus* when exposed to a field collected leaves for 24 hours.

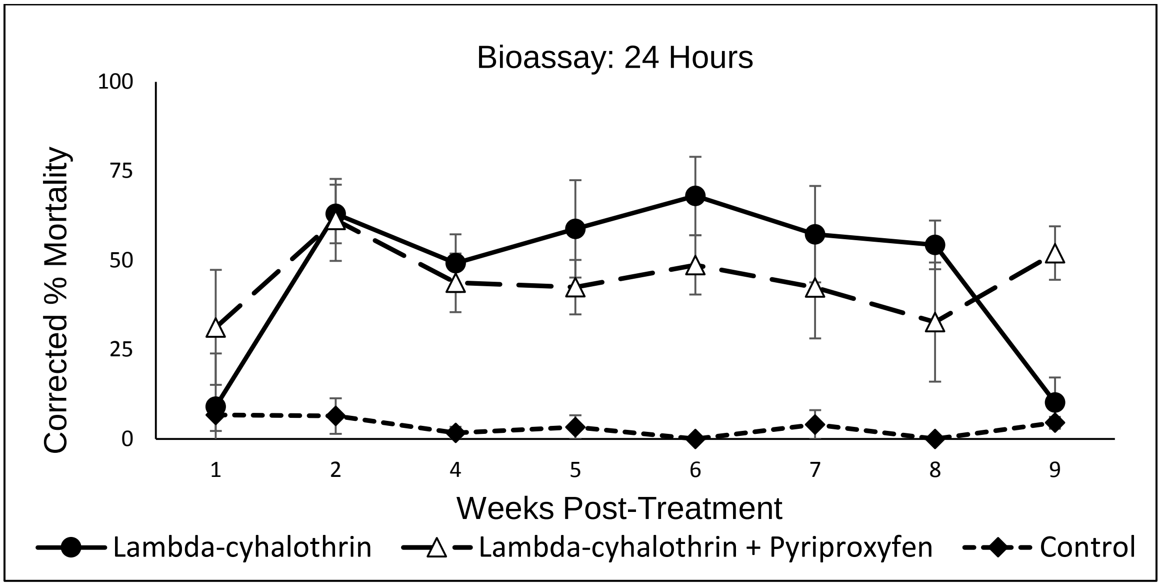


Figure 1.3 Mean (± SEM) corrected percent mortality of lab-reared *Ae. albopictus* when exposed to a field collected leaves for 48 hours.

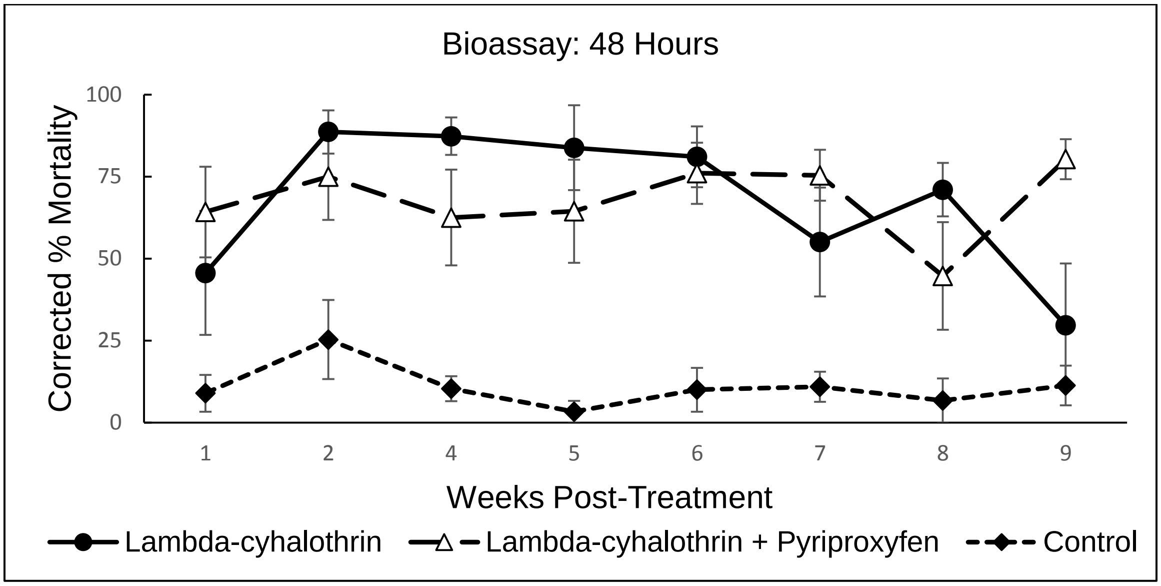


Figure 1.4, Mean (± SEM) larvae collected in ovitraps per week from 30 suburban backyards (6 July – 9 September 2015) from Lexington, KY.

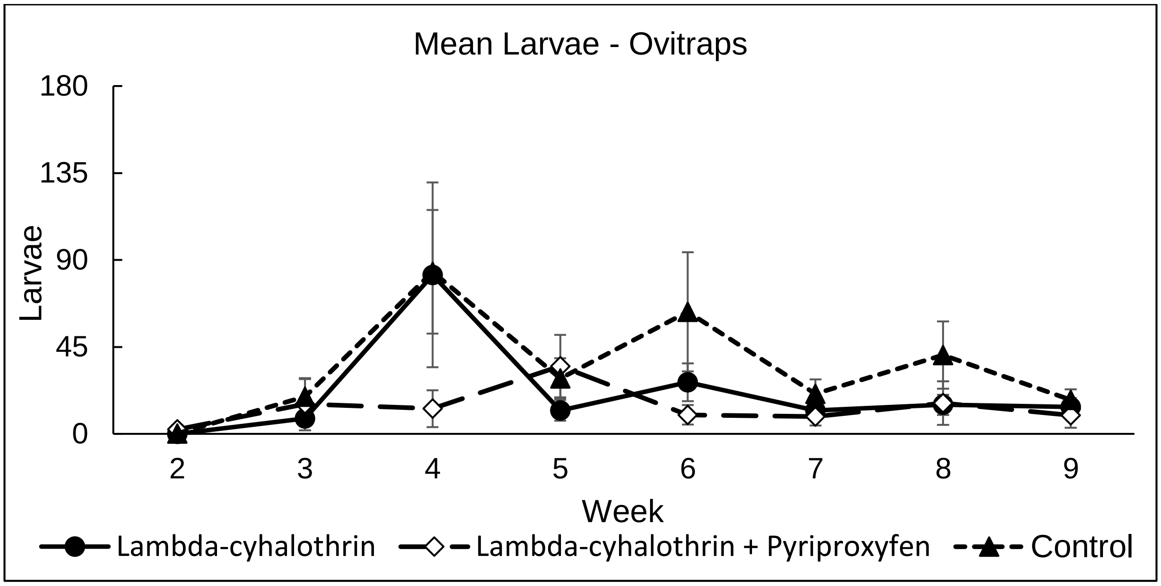


Figure 1.5, Mean (± SEM) mosquitoes collected in both CDC and gravid traps per week from 30 suburban backyards (6 July – 9 September 2015) from Lexington, KY between 1600 and 1000 hours.

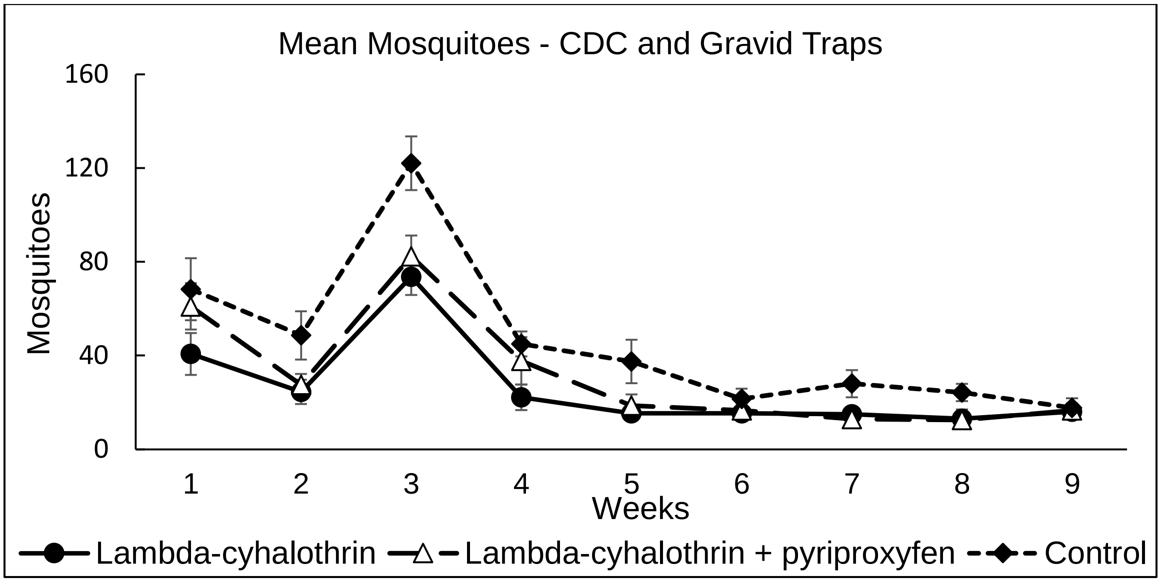


Figure 1.6, Mean (± SEM) mosquitoes collected in CDC traps per week from 30 suburban backyards (6 July – 9 September 2015) from Lexington, KY between 1600 and 1000 hours.

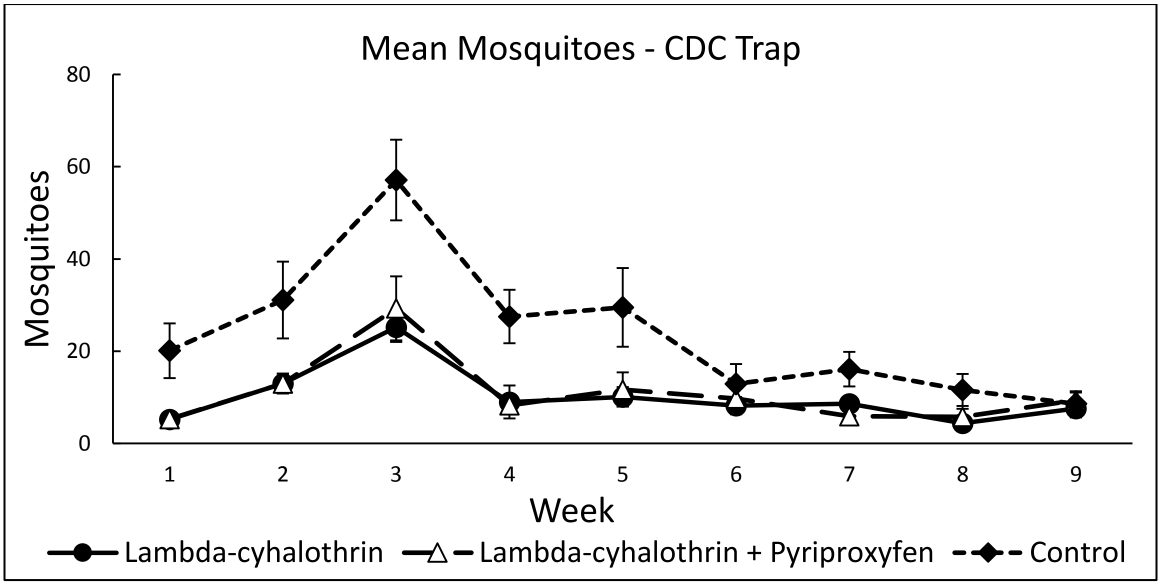


Figure 1.7, Mean (± SEM) mosquitoes collected in gravid traps per week from 30 suburban backyards (6 July – 9 September 2015) from Lexington, KY between 1600 and 1000 hours.

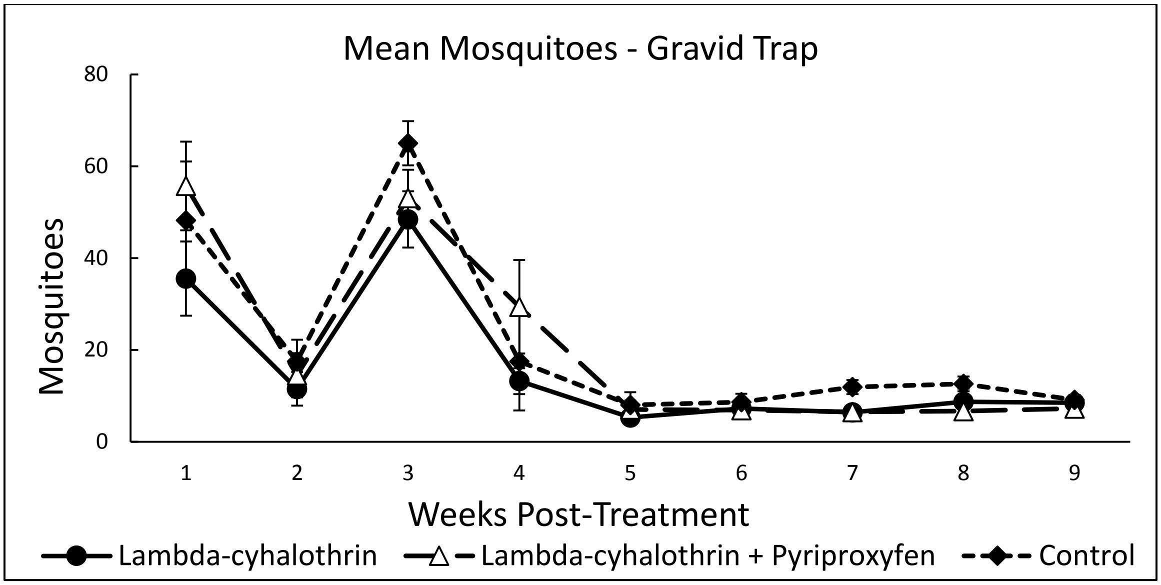


Figure 1.8, Mean (± SEM) *Aedes* and *Ochlerotatus* mosquitoes collected per week from 30 suburban backyards (6 July – 9 September 2015) from Lexington, KY between 1600 and 1000 hours.

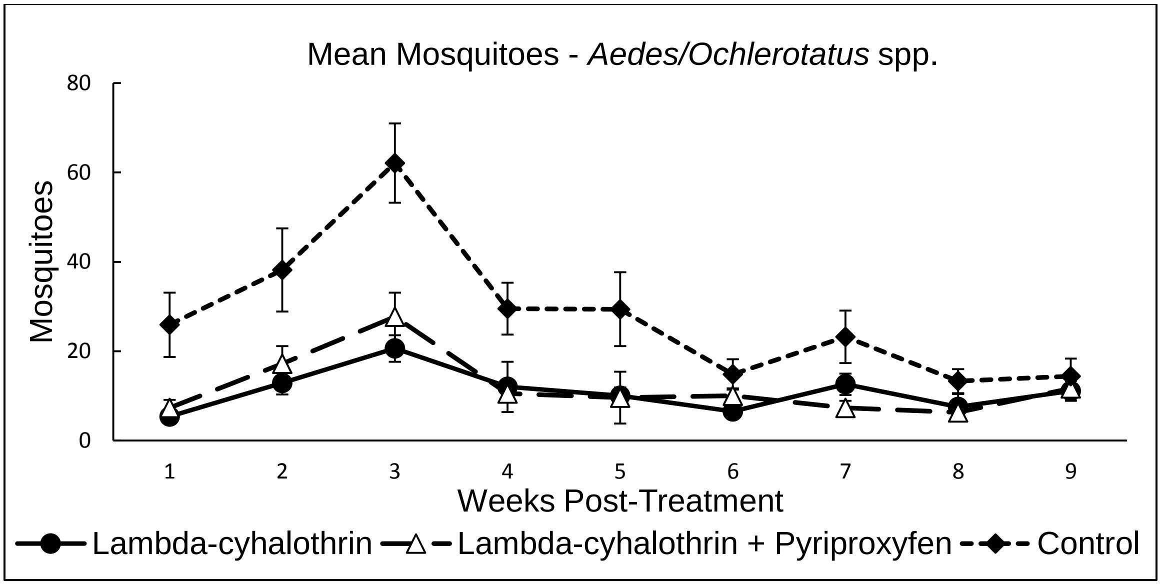


Figure 1.9, Mean (± SEM) *Culex* mosquitoes caught in CDC and gravid traps per week from 30 suburban backyards (6 July – 9 September 2015) from Lexington, KY between 1600 and 1000 hours.

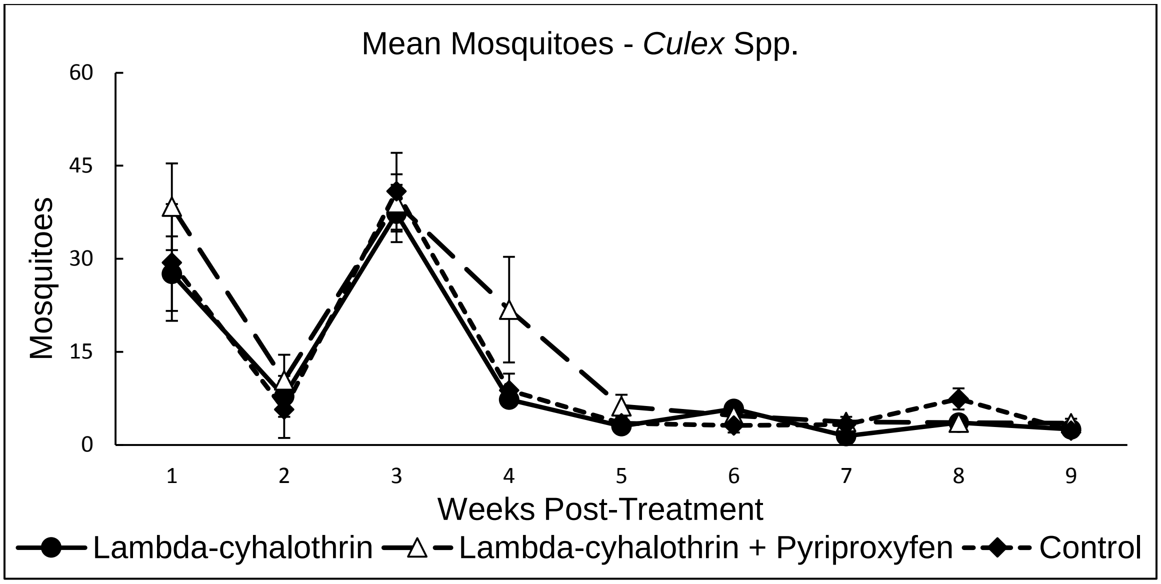
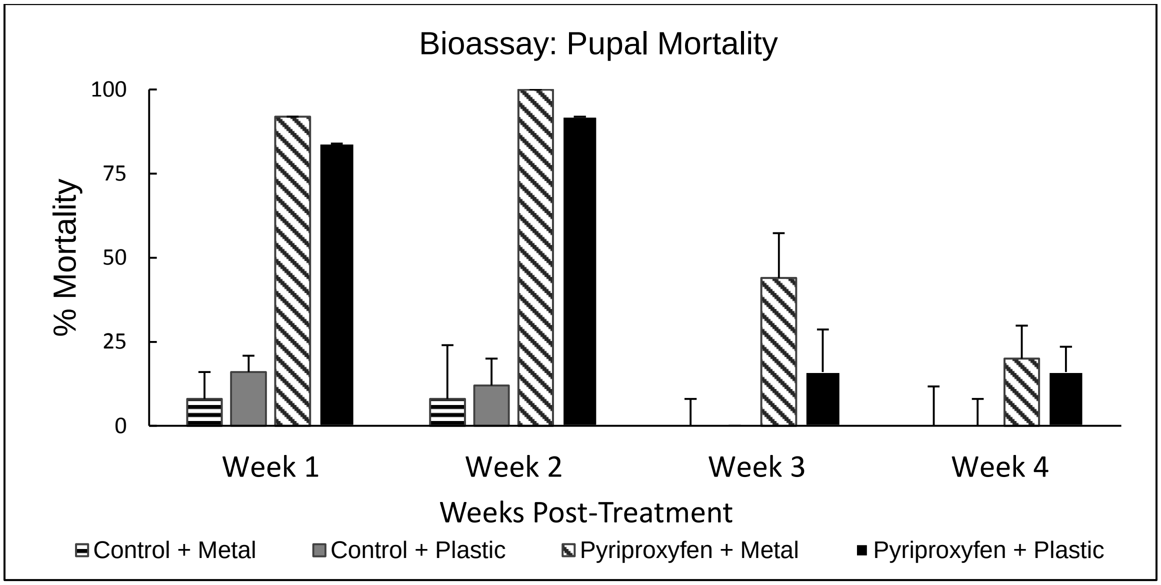


Figure 1.10, Mean (± SEM) pupal mortality of *Ae. albopictus* when exposed to water samples from pyriproxyfen treated containers in a suburban backyard from August 8 – September 9, 2016.



CHAPTER 2

Evaluation of the Addition of Pyriproxyfen to the Synthetic Pyrethroid, Lambda-cyhalothrin for Mosquito Control when Applied with a Truck-mounted Sprayer along Tree-lines

Introduction

Mosquito control professionals rely primarily on ULV sprays applied by truck through residential areas at the municipal level, and with the use of residual perimeter sprays applied with a backpack sprayer by private pest control services. While ULV space sprays are important tools for mosquito control, their efficacy is limited by a number of factors including the need for the insect to come into physical contact with the insecticide droplets while they are still suspended in the air, which in turn necessitates the need to spray at a time of day when the targeted mosquitoes are flying (Bonds 2012). As these sprays are not residual, they must be repeated throughout the mosquito season and their application can be costly for the responsible agencies. Residual sprays are also effective and for weeks at a time, but are only known to protect the parcel of land to which they are applied and because their application is performed with a backpack mist sprayer it is not a realistic method to apply to large areas such as the perimeters of parks, cemeteries, or horse farms. Some options for treating large areas managed at a municipal level or above would include aerial spraying or the use of a truck-mounted mist sprayer.

The use of the synthetic pyrethroid, lambda-cyhalothrin applied as a residual perimeter spray to foliage combines high mortality, with long lasting duration in many different field environments (Trout et al. 2007; Cilek et al. 2008; Britch et al. 2009; Li et al. 2014; Muzari et al. 2014). This treatment has not been tested however, with the use of a truck-mounted mist sprayer. The benefits of this mode of application would include the ability to join the large-scale treatment called for by public health officials and land managers responsible for larger parcels of land than an individual homeowner, with the long lasting effects of a residual perimeter spray. This study will continue evaluation of the addition of pyriproxyfen with lambda-cyhalothrin as performed in Chapter 1, but will test a new application method by utilizing a truck mounted sprayer to treat larger areas than have been possible in the past. I hypothesize that targeting a larger area will better capture the effects of autodissemination (Itoh 1994; Chism and Apperson 2003; Dantur Juri et al. 2013; Suman et al. 2014), as well as the lowered fecundity of female mosquitoes exposed to pyriproxyfen before blood meals (Itoh et al. 1994).

Methods and Materials

Three locations were selected in the summer of 2016; two horse farms, Werkway Stables Inc. of Scott County, KY and Claiborne Farm of Bourbon County, KY and the Lexington Cemetery of Fayette County, KY. The criteria for site selection were that the properties needed to have tree lines with dense perimeter vegetation, easily accessible by a mid-size pick-up truck and to remain available for weekly surveillance throughout the 8-week sampling period. Both of the horse farms featured long tree-lines with dense shrubs, comprised mostly of Japanese honeysuckle (*Lonicera japonica* Thunberg), edging up to the pasture fences. The tree species along the fence lines of the horse farms include *Prunus serotina* Ehrhart, *Celtis occidentalis* Linnaeus, and *Robinia pseudoacacia* L. The Lexington Cemetery had undeveloped areas where around the perimeter grows dense pine (*Pinus Strobus* spp. L.) and Japanese honeysuckle vegetation.

Three treatments were used and replicated 5 times: lambda-cyhalothrin (Demand® CS, 6.25ml/l, Syngenta Crop Protection, Greensboro, NC), lambda-cyhalothrin (6.25 ml/l) + pyriproxyfen (Archer® 7.81ml/l, Syngenta Crop Protection, Greensboro, NC), and a water control. Treatments were applied July 11-12, 2016. At each of the three sites, five, 92 m long stretches of perimeter were plotted and randomly assigned to one of three treatments. Between each treatment plot, a 92 m stretch of perimeter was left as a spacer between treatments. The treatments were applied when the vegetation was dry from any dew or rain and when there was little to no wind. Application was performed using the Boss ATV engine driven mist sprayer (A-1 Mist Sprayers Resources Inc., Ponca, NE), mounted on the bed of a mid-size pick-up.

Mosquito Surveillance. Following treatment, all properties were sampled for mosquitoes on weeknights for 8 weeks (July 18, 2016 through September 9, 2016). The fifth week post-treatment was not sampled, due to severe weather. Sampling methods performed at each property: (1) Centers for Disease Control (CDC) miniature light traps (Model 512, John W. Hock, Gainseville, FL), (2) CDC gravid traps (Model 1712, John W. Hock, Gainesville, FL), (3) Ovitraps made from black plastic, 12cm diameter, (4) Finally, water samples were collected from cups baited with distilled water and bioassays were performed with second instar, lab-reared *Ae. albopictus* larvae. After the mosquitoes were collected from the field, they were placed in a cooler and taken back to the lab, where they were frozen, identified to species, and counted.

CDC miniature light traps were placed in the center of each treated plot between 1600 and 1000, hanging just inside, or on the fence-line. They were baited with a light (Type: CM-47, 150 mAmp/hr) and approximately 2.3 kg of pelleted dry ice. The dry ice was packed into 1.89 L coolers ("Contour™ 0.5" Gallon, Igloo Products Corps., Houston, TX). CO_2 was able to escape through the opened top spout, two holes drilled into the sides, and one hole drilled in the bottom into which a clear Tyson tube (0.5" OD x 3/8" ID Vinyl Tubing, Model 089) cut to 0.6 m was inserted to deliver the flow of CO_2 directly towards the top of the trap. The CDC light traps were used primarily to attract female mosquitoes questing for a blood meal and will also catch a higher percentage of *Aedes/Ochlerotatus* spp. than *Culex* spp. or others (Trout et al. 2007).

Gravid traps were set out directly under the CDC miniature light traps between 1600 and 1000 and were baited with 4 l of grass-infused water. These traps are designed to catch ovipositing females. The water infusion used in the gravid traps will generally attract a higher percentage of *Culex* spp. The grass-infused water was made by mixing 19 l of water with 100 g of cat chow (Friskies®, Nestlé Purina Petcare, St. Louis, MO, USA), approximately 0.5 g of fescue grass, and 0.5 ml TopFin® Tap Water Aquarium Dechlorinator Water Conditioner (PetSmart Inc., Phoenix, AZ).

Ovitraps were black plastic cups (500 ml) lined with egg paper (76 lb. seed germination paper, Anchor Paper Co., Minneapolis, MN) and filled with the same grass-infused water used in the gravid traps. At each treatment plot, one cup was hung from a branch at 1.5 m. The sentinel cups for bioassays were collected from plastic paint mixing cups (HDX, 1-qt. Multi-Mix Pail, Model #2M3) that were filled with distilled water. Collections from the ovitraps and sentinel cups were taken weekly.

Laboratory Bioassays. The bioassays used 100 ml glass vials, filled with water samples from the sentinel cups in the field, as well as 1 ml of liver powder solution to provide a food source to the maturing larvae. Each vial contained five laboratory reared, second instar *Ae. albopictus* larvae and were stored in a growth chamber set at 27°C and 75% R.H. At 24 h intervals, the bioassays were evaluated for pupal mortality, which was determined when mosquito pupae did not respond with movement to stimulation.

Statistical Analysis. All statistical analyses were performed using JMP 12.1 (SAS Institute, 2015). The number of mosquitoes trapped at each location, were normalized using the square-root transformation and analyzed using ANOVA and Tukey-Kramer comparison of means. The model was constructed using both the week and treatment variables as combined effects. Abbott's formula (Abbott, 1925) was used to calculate the percent reduction in the mean number of mosquitoes caught at each location. Abbott's formula:

$$\text{Corrected \%} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) * 100$$

Where n = insect sample, T = treatment, Co = control

Mosquito bioassays from the field were also adjusted, using the Schneider - Orelli correction (Puntener 1981). Schneider - Orelli correction:

$$\text{Corrected \%} = \left(\frac{\text{\% Mortality treated} - \text{\% Mortality control}}{100 - \text{\% Mortality Control}} \right) * 100$$

The field bioassay percent reductions were untransformed and analyzed with Tukey's test. Backyard bioassays were analyzed using a chi-square approximation.

Results and Discussion

Mosquito monitoring. Over the course of the season, 1,907 mosquitoes were collected in the CDC and gravid traps, comprising 20 species and six genera (Table 2.1). *Aedes/Ochlerotatus* spp. accounted for 38% of the total specimens, *Culex* spp. 48%, *Anopheles* spp. 2%, and *Psorophora* spp., *Culiseta* spp., and *Uranotaenia* spp. <1% each. Due to being badly damaged by passing through the fans of the traps, 7% of the mosquitoes were unable to be identified. Males made up 2% of the total collection, which were excluded from further measures of analysis.

CDC traps collected 49% of the specimens and gravid traps 51%. The CDC trap data shows that 50% of specimens caught were *Culex* spp., 34% were *Aedes/Ochlerotatus* spp., and 6% *Anopheles* spp. The same breakdown of gravid traps shows 45% were *Aedes/Ochlerotatus* spp., 46% *Culex* spp., and 2% *Anopheles* spp.

Surprisingly, 58% of all *Aedes/Ochlerotatus* spp. were caught in CDC traps, while only 49% of all *Culex* spp. were caught in gravid traps. This is a reversal of the common trend, in which gravid traps (particularly those baited with grass water) will attract and capture primarily *Culex* spp. and CDC traps will collect a higher percentage of *Aedes* spp. The inclusion of the light in this set-up, as opposed to only using a CO_2 bait, may have attracted a greater number of *Culex* spp. particularly at these locations, alternate light sources would not have been nearby to compete with the traps.

The top three most abundant species from this sampling period were *Ae. albopictus* (31%), *Cx. pipiens* (29%), and *Cx. erraticus* (12%). A species of particular importance to public health, *Culex nigripalpus Theobald*, was found in high numbers (5%) at the Scott Co. location. This is an unusual occurrence in Kentucky this far north. In mosquito surveillance of Jefferson Co., KY by Covell (1968) and in the sampling conducted in Fayette Co., KY by Trout et al. (2007) in the summers of 2005 and 2006, no *Cx. nigripalpus* were found. This is also an increase in the number caught from the previous season's surveillance (Chapter 1).

Ovitraps and bioassays of field-collected water samples. Collection of eggs was unsuccessful, as the containers used were damaged or drained, by a combination of interference from animals in the field and the heavy storms which came during the season. The same issue was seen with the sentinel cups, however, three weeks of sampling was conducted. The bioassay results did not show significant effects, with eight week post-treatment cumulative reductions for samples taken from lambda-cyhalothrin treatments and lambda-cyhalothrin + pyriproxyfen being 1.3% and 6.7%, respectively (Figure 1.1).

Trap analysis. Over the course of the study, the uncorrected mean number of mosquitoes caught at each plot/week was 16 $\pm$ 2 from plots treated with lambda-cyhalothrin, 15 $\pm$ 3 from plots at which pyriproxyfen was added, and 24 $\pm$ 3 from plots in the control. The percent reduction in plots treated with lambda-cyhalothrin was 40.4% 4 weeks post-treatment, 41.5% 6 weeks post-treatment, and 40.3% 8 weeks post-treatment. From plots in the lambda-cyhalothrin + pyriproxyfen treatments the percent reduction was 33.9% 4 weeks post-treatment, 38.4% 6 weeks post-treatment, and 34.8% 8 weeks post-treatment. The combined CDC and gravid trap (Figure 2.2) results showed significant treatment week interaction (F = 2.89; df = 8, 104; P = 0.0063), week (F = 2.48; df = 6; P = 0.0282), and treatment (F = 4.1; df = 2; P = 0.0195) effects. The Tukey's test showed significant difference only between the lambda-cyhalothrin + pyriproxyfen compared to the control (T = -2.67; df = 96; P =

0.0237). This result differed from the trend seen in the previous summer's data in which no significant difference was seen between treatments. Performing the Tukey's test by-week however, did not show a significant effect of even cyhalothrin + pyriproxyfen against the control past the first week of treatment.

To better understand the dynamics of the suppression seen from the treatments, the results were further broken down by trap type. Neither of the trap types showed a significant treatment effect. The results of the CDC traps alone (Figure 2.3) showed a significant treatment week interaction effect (F = 2.45; df = 8, 104; P = 0.0185), as well as significant week effects (F = 2.45; df = 8, 104; P = 0188), but not a significant treatment effect. The mean number of mosquitoes caught each week in the CDC traps was 7 $\pm$ 1.51 in lambda-cyhalothrin treated plots, 9 $\pm$ 2.76 in lambda-cyhalothrin + pyriproxyfen treated plots, and 11 $\pm$ 1.73 in control plots. The gravid traps (Figure 2.4) also showed a significant difference in treatment week interaction (F = 2.57; df = 8, 104; P = 0.0138) and week effect (F = 2.47; df = 6; P = 0.0291). The Tukey's test showed a significant difference between the lambda-cyhalothrin + pyriproxyfen and control treatments (T = -2.4; df = 96; P = 0.0476). The mean number of mosquitoes caught in the gravid traps each week was 8 $\pm$ 2 in the lambda-cyhalothrin treatment, 6 $\pm$ 1 in the lambda-cyhalothrin + pyriproxyfen treatment, and 13 $\pm$ 3 in the control. As the gravid traps will attract mosquitoes looking for oviposition habitat, this result suggests that although suppression may have been seen, it was in mosquitoes who had already blood fed and were looking for breeding sites.

Analysis of the genera showed that the *Aedes* spp. (Figure 2.5) has a significant treatment week interaction effect (F = 3.16; df = 8,104; P = 0.0032), week effect (F = 2.83; df = 6; P = 0.0142), and treatment (F = 4.15; df = 2; P = 0.0187). The Tukey's test showed a significant difference between the number of mosquitoes caught in lambda-cyhalothrin treated plots and control plots (T = -2.73; df = 96; P = 0.0206). The average number of mosquitoes caught in each trap per week was 3.6 $\pm$ 0.66 in lambda-cyhalothrin plots, 5.7 $\pm$ 1.68 in the lambda-cyhalothrin + pyriproxyfen plots, and 11.8 $\pm$ 3.4 in the control plots. The cumulative percent reductions 4, 6, and 8 weeks post-treatment were 43.7%, 41.4%, and 40.1%, respectively in the lambda-cyhalothrin treatments. The lambda-cyhalothrin + pyriproxyfen treatments showed cumulative reductions of 33.3% at 4 weeks post-treatment, 36.5% at 6 weeks, and 32.6% at 8 weeks. This result was akin to those seen in the previous season's results, with the addition of pyriproxyfen not showing a significant increase in suppression compared to the conventional pyrethroid only treatment.

Conclusion

The application of a residual perimeter spray along tree lines using a truck mounted sprayer did not show the same levels of mosquito suppression as has been seen in treatments applied with a backpack sprayer to small-scale residences. There was a significant effect of the treatment, though the percent reduction was less than what might be expected given the levels of control obtained in previous studies (Trout et al, 2007; Cilek et al., 2008; Britch et al., 2009). Though there was not significant control of *Culex* spp., which were the target group due to their role as vectors of viral encephalitis in Kentucky, the truck mounted sprayer shows potential for its ability to be used in the application of large-scale perimeter sprays. One of the potential weaknesses of this study's design was the application of the treatments in linear strips, as opposed to a true perimeter. Without a true perimeter the entry of mosquitoes unlikely to have ever contacted the treated vegetation will be higher, and perhaps may have replaced any suppression that would have been seen. It is possible, particularly for species such as *Ae. vexans*, which travel greater distances than a species such as *Ae. albopictus* to reach their blood-meals, that failing to apply a complete perimeter treatment led to insects being able to fairly easily fly into the trapping area from the adjacent, untreated "spacer" plots.

Another potential issue with the application was that the volume of product applied may not have provided the same level of coverage as is put out with a backpack sprayer. Due to scheduling restraints, we were not able to test the calibration of the machine with the use of water sensitive paper to fully measure the depth of penetration into the foliage, and so while the recommended settings were used, measurements of percent cover of foliage were not made. The swath width was greater (~2 m) than what is obtained with a backpack sprayer (~1 m), and so an equivalent volume of product may have offered insufficient coverage of insecticide.

An unexpected observation of this study was the relatively high number of *Cx. nigripalpus*. Not only is this species unusual this far north, but it is also an important vector of St. Louis Encephalitis (SLE), and a buildup in the population often proceeds outbreaks of this potentially lethal disease. Outbreaks of SLE have been historically recorded in Louisville and in the Ohio River Basin, and mosquito surveillance has been identified as an important component of epidemic predictability (Day, 2001). As such, the presence of this species will need to monitor in subsequent years.

Table 2.1 Total mosquitoes collected from field plots (18 July – 9 September, 2016) from three locations in Central, KY between 1600 and 1000 hours.

Species	CDC	Gravid	Total	Total %
Ae albopictus	173	411	584	30.62
Cx pipiens	186	360	546	28.63
Cx erraticus	150	69	219	11.48
Ae vexans	119	8	127	6.66
Cx nigripalpus	92	11	103	5.40
Cx spp.*	34	3	37	1.94
An quadrimaculatus	19	17	36	1.89
An punctipennis	22	0	22	1.15
Oc japonicus	2	7	9	0.47
Oc sollicitans	8	1	9	0.47
Ae spp.	4	4	8	0.42
An spp.	8	0	8	0.42
Oc triseriatus	2	3	5	0.26
Cx tarsalis	4	0	4	0.21
Ps columbiae	2	1	3	0.16
Ps cyanescens	3	0	3	0.16
Ur sappharina	3	0	3	0.16
An perplexans	2	0	2	0.10
An crucians	1	0	1	0.05
Cs melanura	1	0	1	0.05
Oc cinereus	1	0	1	0.05
Oc trivittatus	5	0	5	0.26
Ps howardii	1	0	1	0.05
Ps spp.	1	0	1	0.05
Unknown	71	64	135	7.08
Male	22	12	34	1.78
Total	936	971	1907	100

Figure 2.1 Mean (± SEM) percent mortality of lab-reared *Ae. albopictus* pupae when exposed to field collected water samples from sentinel cups (18 July – 9 September, 2016) from three locations in Central, KY.

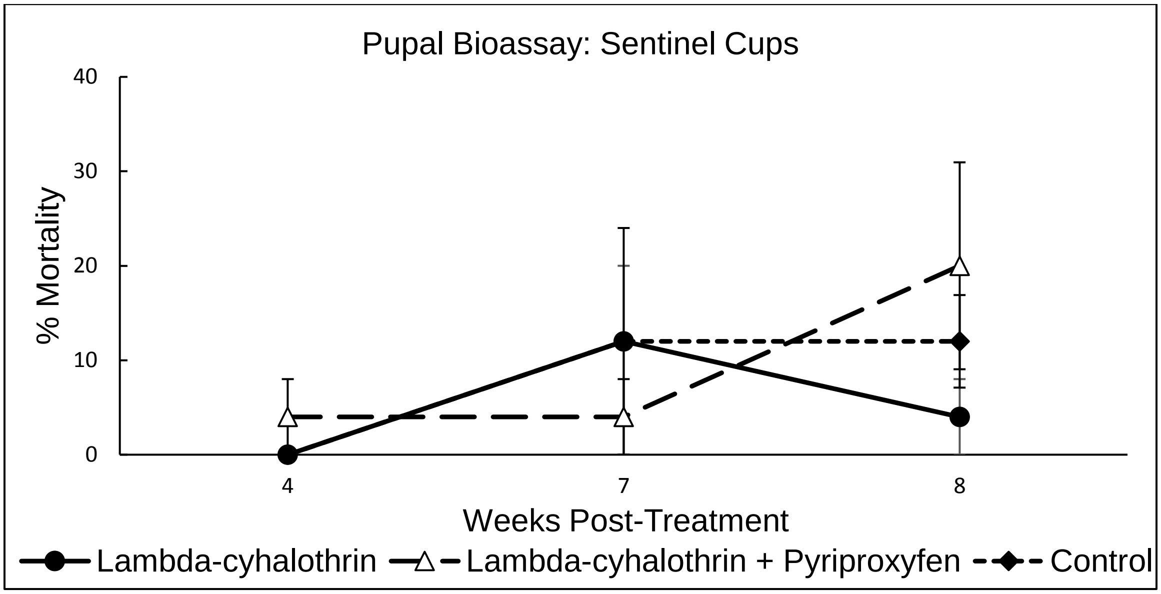


Figure 2.2 Mean (± SEM) mosquitoes collected in both CDC and gravid traps per week (18 July – 9 September, 2016) from three locations in Central, KY between 1600 and 1000 hours.

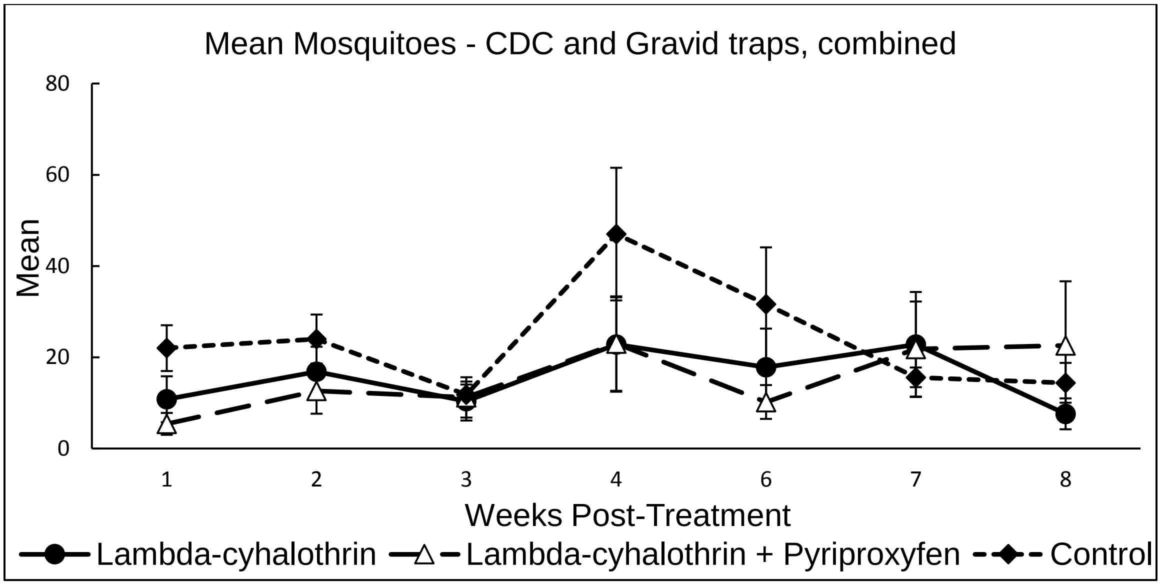


Figure 2.3, Mean (± SEM) mosquitoes collected in CDC traps per week (18 July – 9 September, 2016) from three locations in Central, KY between 1600 and 1000 hours.

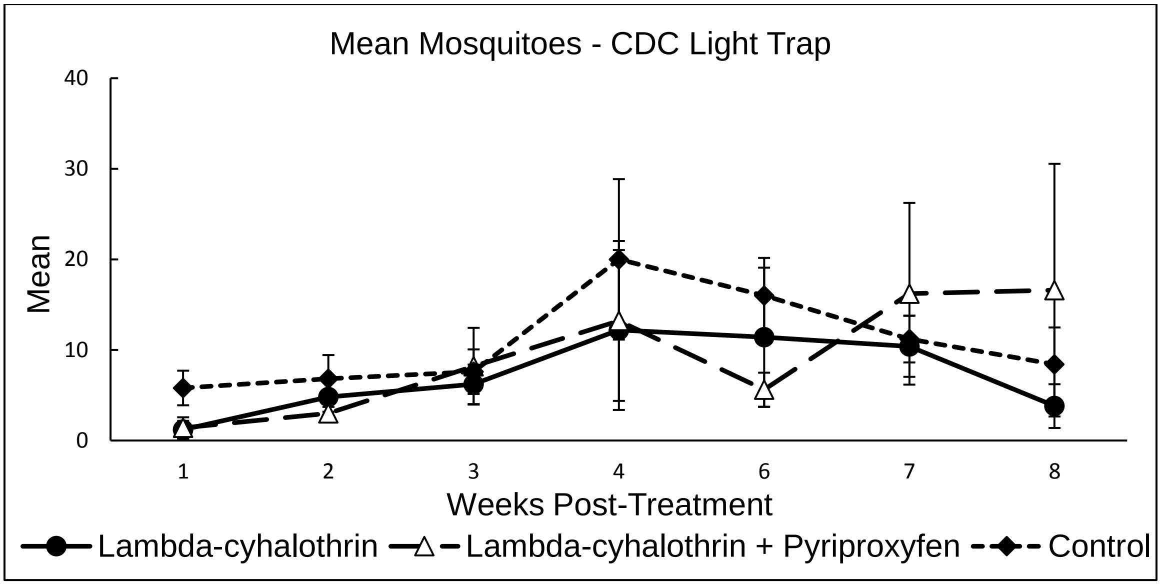


Figure 2.4, Mean (± SEM) mosquitoes collected in gravid traps per week (18 July – 9 September, 2016) from three locations in Central, KY between 1600 and 1000 hours.

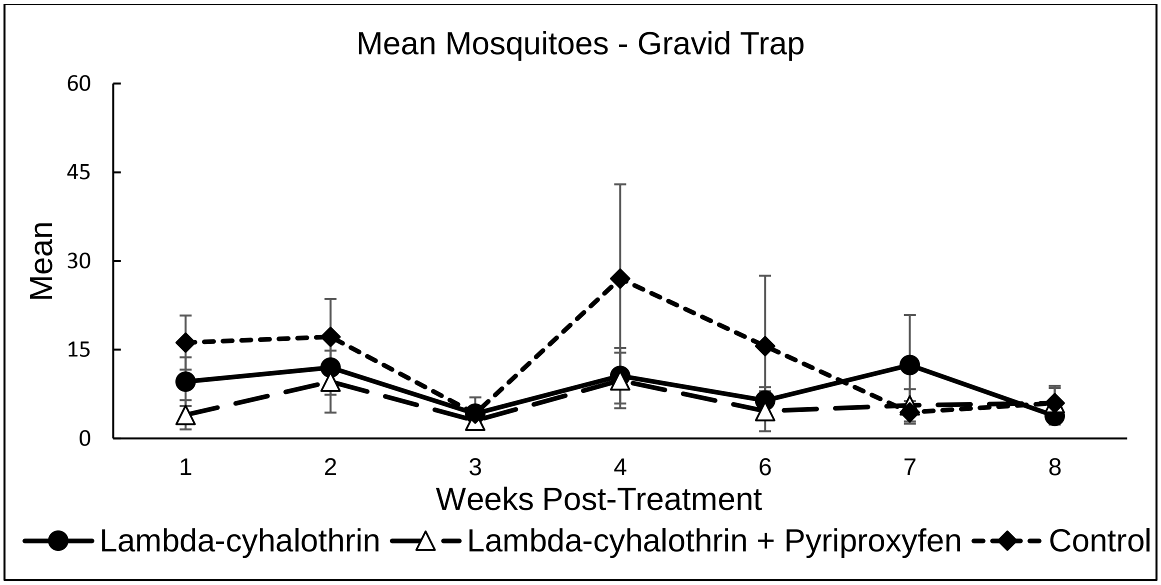


Figure 2.5 Mean (± SEM) *Aedes* and *Ochlerotatus* mosquitoes collected per week (18 July – 9 September, 2016) from three locations in Central, KY between 1600 and 1000 hours.

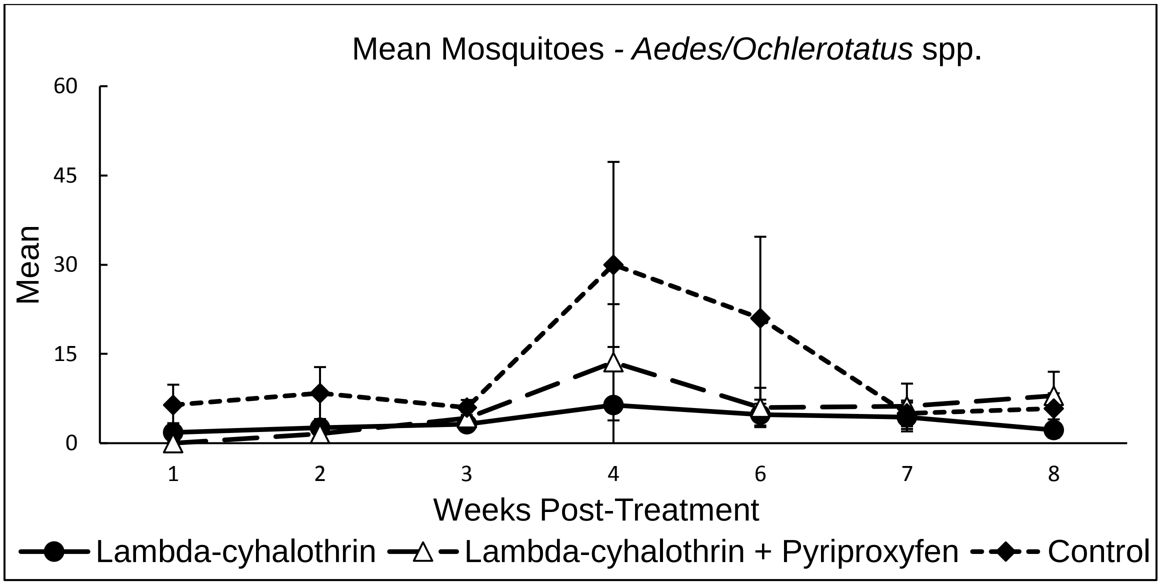
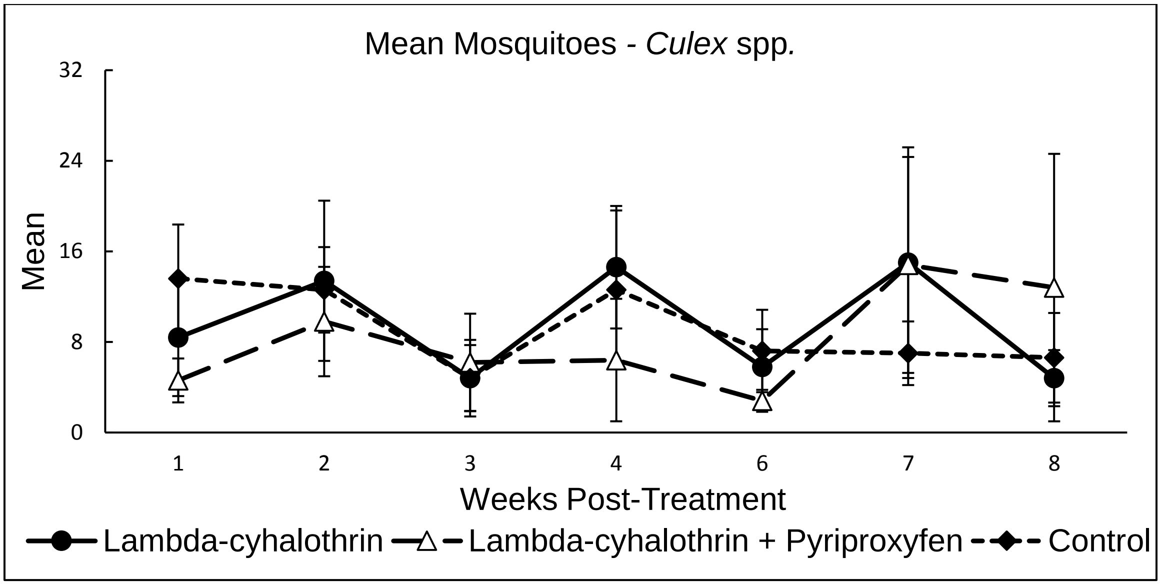


Figure 2.6 Mean (± SEM) *Culex* mosquitoes caught in CDC and gravid traps per week (18 July – 9 September, 2016) from three locations in Central, KY between 1600 and 1000 hours.



APPENDIX I

2015 Survey and Results administered to assess Homeowner Satisfaction

Initial Survey Results – Administered 2 weeks pre-treatment:

1. How much time do you spend in your backyard per day?

<1 hr 2-4hrs 4-8hrs >8hrs

Treatment	N	<1hr	2-4hrs	4-8hrs	>8hrs
Lambda-cyhalothrin	9	56%	44%	0%	0%
Lambda-cyhalothrin + Pyriproxyfen	9	33%	66%	0%	0%
Control	10	40%	60%	0%	0%
Total Combined	28	63%	89%	0%	0%

2. When are you most likely to be outdoors?

Early morning Noon Afternoon Evening

Treatment	N	Early Morning	Noon	Afternoon	Evening
Lambda-cyhalothrin	9	22%	11%	56%	56%
Lambda-cyhalothrin + Pyriproxyfen	10	30%	0%	40%	90%
Control	10	30%	0%	40%	90%
Total Combined	29	28%	3%	45%	79%

3. Mosquitoes are more likely to bite me than most other people.

Strongly agree Agree Disagree Strongly Disagree N/A

Treatment	N	Strongly Agree	Agree	Disagree	Strongly Disagree	N/A
Lambda-cyhalothrin	10	10%	30%	60%	0%	0%
Lambda-cyhalothrin + Pyriproxyfen	10	40%	30%	20%	0%	10%
Control	10	50%	10%	20%	0%	30%
Total Combined	30	33%	23%	33%	0%	13%

4. Mosquitoes populations have limited my backyard experience.

Strongly agree Agree Disagree Strongly Disagree N/A

Treatment	N	Strongly Agree	Agree	Disagree	Strongly Disagree	N/A
Lambda-cyhalothrin	10	30%	30%	40%	0%	0%
Lambda-cyhalothrin + Pyriproxyfen	10	10%	50%	30%	10%	0%
Control	10	20%	60%	20%	0%	0%
Total Combined	30	20%	47%	30%	3%	0%

5. I worry about the health threat from mosquitoes.

Strongly agree   Agree   Disagree   Strongly Disagree   N/A

Treatment	N	Strongly Agree	Agree	Disagree	Strongly Disagree	N/A
Lambda-cyhalothrin	10	20%	50%	30%	0%	0%
Lambda-cyhalothrin + Pyriproxyfen	10	10%	60%	20%	0%	10%
Control	10	20%	70%	0%	0%	10%
Total Combined	30	17%	60%	17%	0%	7%

6. In your opinion, is there a mosquito problem in Lexington, KY?

Yes   No   Not Sure

Treatment	N	Yes	No	Not Sure
Lambda-cyhalothrin	10	40%	0%	60%
Lambda-cyhalothrin + Pyriproxyfen	10	60%	10%	30%
Control	10	80%	0%	20%
Total Combined	30	60%	3%	37%

7. In your opinion, has the mosquito population gotten better, worse, or has it remained the same in the past 5 years?

Better   Worse   Same

Treatment	N	Better	Worse	Same
Lambda-cyhalothrin	10	0%	20%	80%
Lambda-cyhalothrin + Pyriproxyfen	10	10%	40%	50%
Control	10	0%	70%	30%
Total Combined	30	3%	43%	53%

8. Where do you believe mosquitoes in your backyard breed?

a. Standing water off your property

b. Standing water on your property

c. Standing water on and off your property

d. Lakes and rivers nearby

e. Other____________________________

f. Not Sure

Treatment	N	a.	b.	c.	d.	e.	f.
Lambda-cyhalothrin	10	10%	0%	50%	10%	10%	40%
Lambda-cyhalothrin + Pyriproxyfen	10	60%	0%	40%	20%	20%	0%
Control	10	50%	0%	30%	10%	20%	10%
Total Combined	30	40%	0%	40%	13%	17%	17%

9. How many mosquito bites per night do you believe indicates a mosquito problem in your backyard? ______

Treatment	N	Mean
Lambda-cyhalothrin	10	4.40
Lambda-cyhalothrin + Pyriproxyfen	9	5.3*
Control	10	4
Total Combined	29	4.4

* An outlier of 72 was removed and the mean recalculated.

10. Have you ever contacted the KY Division of Environmental Assistance or the Fayette Country Urban Government about mosquito control?

Yes No Not Sure

Treatment	N	Yes	No	Not Sure
Lambda-cyhalothrin	10	0%	90%	10%
Lambda-cyhalothrin + Pyriproxyfen	10	0%	90%	10%
Control	10	0%	90%	10%
Total Combined	30	0%	90%	10%

11. Do you think the KY Division of Environmental Assistance or the Fayette Country Urban Government should do more in controlling mosquitoes?

Yes No Not Sure

Treatment	N	Yes	No	Not Sure
Lambda-cyhalothrin	10	30%	10%	60%
Lambda-cyhalothrin + Pyriproxyfen	10	20%	10%	70%
Control	10	70%	0%	30%
Total Combined	30	40%	7%	53%

12. Would you be willing to pay more in property taxes for better mosquito control?

Yes No Not Sure

Treatment	N	Yes	No	Not Sure
Lambda-cyhalothrin	10	0%	70%	30%
Lambda-cyhalothrin + Pyriproxyfen	10	20%	40%	40%
Control	10	30%	20%	50%
Total Combined	30	17%	43%	40%

13. Do you regularly purchase insecticides to control mosquitoes in your yard?

Yes No Not Sure

Treatment	N	Yes	No	Not Sure
Lambda-cyhalothrin	10	20%	80%	0%
Lambda-cyhalothrin + Pyriproxyfen	10	40%	60%	0%
Control	10	20%	80%	0%
Total Combined	30	27%	73%	0%

14. Approximately how much money do you spend on insecticides for mosquito control each summer? ________

Treatment	N	$0	$0 - 20	$20-40	>$40
Lambda-cyhalothrin	9	88%	0%	22%	0%
Lambda-cyhalothrin + Pyriproxyfen	7	67%	14%	29%	0%
Control	8	38%	38%	25%	0%
Total Combined	24	80%	22%	32%	0%

15. How confident are you that a spray applied to your perimeter foliage will substantially reduce mosquitoes in your backyard for a month or more?

Not confident Somewhat Very confident No Opinion

Treatment	N	Not Confident	Somewhat	Very Confident	Unsure	No Opinion
Lambda-cyhalothrin	10	10%	70%	0%	20%	0%
Lambda-cyhalothrin + Pyriproxyfen	10	0%	60%	0%	40%	0%
Control	10	10%	50%	20%	0%	20%
Total Combined	30	7%	60%	7%	20%	7%

2015 Survey and Results administered to assess Homeowner satisfaction.

Midtrial Survey Results – Administered 4 weeks post-treatment:

1. Has the treatment reduced mosquito populations to your satisfaction?

Yes No Not Sure

Treatment	N	Yes	No	Not Sure
Lambda-cyhalothrin	10	80%	10%	10%
Lambda-cyhalothrin + Pyriproxyfen	7	29%	29%	43%
Control	8	25%	38%	38%
Total Combined	25	48%	24%	28%

2. I would recommend this treatment to a friend or family member.

Strongly Agree Agree Neutral Disagree Strongly Disagree Unsure

Treatment	N	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	Unsure
Lambda-cyhalothrin	10	10%	70%	10%	10%	0%	0%
Lambda-cyhalothrin + Pyriproxyfen	6	17%	33%	29%	0%	0%	17%
Control	8	0%	38%	25%	13%	0%	25%
Total Combined	24	8%	50%	21%	8%	0%	13%

The next three questions (3, 4, & 5) use the ranking system copied from your magnet (weekly log), please note which rank best correlates with the statements. Please answer these questions based only on your backyard experience during the month of July.

Rank	**Behavior**
1:	**We/I did not notice mosquitoes**
2:	**We noticed or were bitten by mosquitoes but not enough to use repellents or avoid being outdoors.**
3:	**At least some of us were bothered by mosquitoes enough to use repellents or avoid being outdoors.**
4:	**Mosquitoes were very noticeable and were a definite annoyance for most of July.**
5:	**Mosquitoes have been very bad during the month of July.**

3. I feel the ranking that best describes the mosquito population, since the treatment and <u>for me personally</u>, is ______ (rank).

Treatment	N	1	2	3	4	5
Lambda-cyhalothrin	10	30%	60%	0%	0%	10%
Lambda-cyhalothrin + Pyriproxyfen	7	57%	14%	14%	14%	0%
Control	8	0%	50%	25%	13%	13%
Total Combined	25	28%	44%	12%	8%	8%

4. I feel the ranking that best describes the mosquito population, since the treatment and <u>for the most mosquito-sensitive person in my household</u>, is ______ (rank).

Treatment	N	1	2	3	4	5
Lambda-cyhalothrin	10	30%	50%	10%	0%	10%
Lambda-cyhalothrin + Pyriproxyfen	7	57%	14%	14%	14%	0%
Control	8	0%	25%	50%	13%	13%
Total Combined	25	28%	32%	24%	8%	8%

5. Normally (i.e. without the treatment) I believe my typical mosquito population at this point in the last season, is __________ (rank).

Treatment	N	1	2	3	4	5
Lambda-cyhalothrin	10	0%	10%	20%	40%	30%
Lambda-cyhalothrin + Pyriproxyfen	7	0%	14%	43%	29%	14%
Control	8	0%	0%	13%	50%	38%
Total Combined	25	0%	8%	24%	40%	28%

6. Which statement most closely reflects the treatment's effect on your use of your backyard, during the month of July?

A. ___ Mosquitoes were much worse in spite of the treatment

B. ___ Mosquitoes were worse in spite of the treatment

C. ___ Treatment had no effect on mosquitoes

D. ___ The treatment let me be outdoors a little longer

E. ___ The treatment enabled me to be outdoors much longer

Treatment	N	A	B	C	D	E
Lambda-cyhalothrin	10	0%	0%	20%	40%	40%
Lambda-cyhalothrin + Pyriproxyfen	7	0%	14%	29%	29%	29%
Control	8	0%	13%	25%	50%	13%
Total Combined	25	0%	8%	24%	40%	28%

7. If you answered D or E on the previous question, about how much <u>more</u> time did you spend in your backyard (relative to normal mosquito years) because of the treatment?

A. 0 – 25% B. 25 – 50% C. 50 – 100% D. More than double (i.e. > 100%)

E. Did not answer D or E

Treatment	N	A	B	C	D	E	F
Lambda-cyhalothrin	10	50%	20%	10%	0%	20%	0%
Lambda-cyhalothrin + Pyriproxyfen	7	14%	43%	14%	0%	29%	0%
Control	7	43%	14%	14%	0%	14%	0%
Total Combined	24	28%	25%	13%	0%	21%	0%

8. Did you notice any effect of the treatment that you would consider negative?

Yes No Not Sure

Treatment	N	Yes	No	Not Sure
Lambda-cyhalothrin	10	0%	100%	0%
Lambda-cyhalothrin + Pyriproxyfen	7	0%	100%	0%
Control	8	0%	100%	0%
Total Combined	25	0%	100%	0%

9. Based on your experience with this treatment so far, how much would you pay a pest control company to apply the single treatment that you received?

None < $25 $25 – 50 $50 – 75 $75 – 100 > $100

Treatment	N	A	B	C	D	E	F
Lambda-cyhalothrin	10	20%	20%	40%	10%	10%	0%
Lambda-cyhalothrin + Pyriproxyfen	7	43%	14%	0%	29%	14%	0%
Control	8	38%	13%	25%	25%	0%	0%
Total Combined	25	32%	16%	24%	20%	8%	0%

10. Has the sampling procedure/personnel caused any problem? (Yes/No)

Treatment	N	Yes	No
Lambda-cyhalothrin	10	0%	100%
Lambda-cyhalothrin + Pyriproxyfen	7	0%	100%
Control	8	0%	100%
Total Combined	25	0%	100%

2015 Survey and Results administered to assess Homeowner satisfaction.

End of Trial Survey Results – Administered 9 weeks post-treatment:

1. Over the course of the entire study, this treatment suppressed mosquitoes to my satisfaction:

Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	Unsure

Treatment	N	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	Unsure
Lambda-cyhalothrin	3	33%	67%	0%	0%	0%	0%
Lambda-cyhalothrin + Pyriproxyfen	6	33%	50%	0%	17%	0%	0%
Control	5	60%	0%	0%	40%	0%	0%
Total Combined	14	43%	36%	0%	21%	0%	0%

2. The treatment was applied only once, at the beginning of July. Do you feel that this treatment:

A. ___ Adequately controlled mosquitoes in both July and August

B. ___ Controlled mosquitoes in July but not in August

C. ___ Reduced mosquitoes but not enough to justify a professional service

D. ___ Did not reduce mosquitoes noticeably

Treatment	N	A	B	C	D
Lambda-cyhalothrin	3	100%	0%	0%	0%
Lambda-cyhalothrin + Pyriproxyfen	6	50%	17%	17%	17%
Control	5	40%	10%	0%	40%
Total Combined	14	57%	14%	7%	21%

3. Which statement most closely reflects the treatment's effects on your use of your backyard throughout the entire summer?

A. ___ Mosquitoes were worse in spite of the treatment

B. ___ Treatment had no effect on mosquitoes

C. ___ The treatment let me be outdoors a little longer

D. ___ The treatment enabled me to be outdoors much longer

Treatment	N	A	B	C	D
Lambda-cyhalothrin	3	0%	0%	33%	67%
Lambda-cyhalothrin + Pyriproxyfen	6	0%	17%	17%	67%
Control	5	20%	20%	20%	40%
Total Combined	14	7%	14%	21%	64%

4. If you answered C or D above, about how much *more* time were you able to spend in your back yard during the month of August (relative to just prior to the treatment):

0 <1 hr 2-4 hrs 4-8 hrs >8 hrs

Treatment	N	0	<1	2 to 4	4 to 8	>8
Lambda-cyhalothrin	3	0%	0%	67%	0%	33%
Lambda-cyhalothrin + Pyriproxyfen	5	0%	0%	60%	40%	0%
Control	3	0%	0%	67%	33%	0%
Total Combined	11	0%	0%	64%	27%	9%

5. If you answered C or D in question 3, besides reducing mosquito populations, what other benefits of the treatment did you receive [mark all that apply]:

A. ____ Reduced mosquito bites

B. ____ Reduced disease risk from mosquito bites

C. ____ I wasn't as worried about potential harmful effects of mosquitoes

D. ____ Other noxious insects were reduced (spiders, ants, centipedes, etc.).

E. ____ Other benefit not listed. Specify: ___________________

Treatment	N	A	B	C	D	E
Lambda-cyhalothrin	3	100%	33%	0%	33%	0%
Lambda-cyhalothrin + Pyriproxyfen	5	100%	60%	40%	40%	40%
Control	3	100%	100%	33%	67%	0%
Total Combined	11	100%	64%	18%	36%	9%

6. What negative effects of the treatment did you notice in your backyard?

A. ____ Reduced numbers of beneficial insects (ladybugs, dragonflies, fireflies)

B. ____ Odor, staining, or visible residue from the treatment itself

C. ____ Fear/concern about the safety of the treatment

D. ____ Other negative effects: Specify ______________________________

E. ____ No negative effects noted

Treatment	N	A	B	C	D	E
Lambda-cyhalothrin	3	0%	0%	0%	0%	100%
Lambda-cyhalothrin + Pyriproxyfen	5	0%	0%	0%	40%	60%
Control	5	0%	0%	0%	0%	100%
Total Combined	13	0%	0%	0%	15%	85%

7. Compared to previous years, I felt that **this season's** mosquito population was:

Much Worse Worse Normal Better Much Better

Treatment	N	A	B	C	D	E
Lambda-cyhalothrin	3	0%	0%	0%	0%	100%
Lambda-cyhalothrin + Pyriproxyfen	5	0%	0%	0%	40%	60%
Control	5	0%	0%	0%	0%	100%
Total Combined	13	0%	0%	0%	15%	85%

8. Compared to most other people's yards, I believe that the mosquitoes in my backyard are typically:

Much Worse Worse Normal Better Much Better

Treatment	N	Much Worse	Worse	Neutral	Better	Much Better
Lambda-cyhalothrin	3	0%	0%	33%	0%	67%
Lambda-cyhalothrin + Pyriproxyfen	6			67%	0%	33%
Control	5	20%	20%	40%	20%	0%
Total Combined	14	7%	7%	50%	7%	29%

9. Now that you have experienced one season with this treatment, what is the maximum that you would you be willing to pay for a similar treatment next year?

$0 $25 $50 $100 $200

Treatment	N	0	25	50	100	200
Lambda-cyhalothrin	3	0%	0%	67%	33%	0%
Lambda-cyhalothrin + Pyriproxyfen	6	33%	17%	0%	50%	0%
Control	5	20%	20%	40%	20%	0%
Total Combined	14	29%	14%	29%	36%	0%

2015 Homeowner Weekly Log – Pre-treatment and 4, 6, and 8 weeks post-treatment.

<u>Rank</u> <u>Behavior</u>

1. We did not notice any mosquitoes
2. Not enough to use repellents or to avoid outdoors.
3. At least some of us were bothered by mosquitoes to use protective measures, (e.g. repellents) or avoid being outdoors.
4. Mosquitoes were very noticeable and were a definite annoyance most of the week.
5. Mosquitoes were very bad this week.

July 4, 2015

4-Jul	N	1	2	3	4	5
Lambda-cyhalothrin	7	71%	14%	0	14%	0
Lambda-cyhalothrin + Pyriproxyfen	9	56%	33%	0	0	11%
Control	2	0	0	0	50%	50%
Total	18	56%	22%	0	11%	11%

July 25, 2015

25-Jul	N	1	2	3	4	5
Lambda-cyhalothrin	7	43%	43%	0	0	14%
Lambda-cyhalothrin + Pyriproxyfen	9	22%	56%	11%	11%	0
Control	8	0	13%	50%	25%	13%
Total	24	21%	38%	21%	21%	8%

August 8, 2015

8-Aug	N	1	2	3	4	5
Lambda-cyhalothrin	6	33%	33%	33%	0	0
Lambda-cyhalothrin + Pyriproxyfen	10	40%	20%	40%	0	0
Control	8	13%	50%	0	13%	25%
Total	24	29%	33%	25%	4%	8%

August 22, 2015

22-Aug	N	1	2	3	4	5
Lambda-cyhalothrin	7	57%	29%	0	0	14%
Lambda-cyhalothrin + Pyriproxyfen	10	30%	50%	20%	0	0
Control	8	13%	25%	38%	13%	13%
Total	25	32%	36%	20%	4%	8%

REFERENCES

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J Econ* Entomol 18: 265-267.

Ali, A, Nayar J. De X. 1995. Comparative toxicity of selected larvicides and insect growth regulators to a Florida laboratory population of *Aedes albopictus*. *J Am Mosq Control Assoc,* 11:72-76.

Anderson J, Main A. 2006. Importance of Vertical and Horizontal Transmission of West Nile Virus by Culex pipiens in the Northeastern United States. *J Infect Dis,* 194(11):1577-1579.

Arthur F, Liu S, Zhao B, Phillips, T. 2009. Residual efficacy of pyriproxyfen and hydroprene applied to wood, metal and concrete for control of stored-product pests. *Pest Man Sci,* 65(7):791-797.

Axtell, R.1979. Principles of Integrated Pest Management (IPM) in Relation to Mosquito Control. *Mosquito News* 39:709-718.

Bkhache, M, Tmimi, F-Z, Charafeddine, O, Faraj, C, Failloux, A-B, and Sarih, M. 2016. First report of L1014F-*kdr* mutation in *Culex pipiens* complex from Morocco. *Parasites & Vectors* 9:644

Blake, D. 2013. Making money with mosquito control. *Pest Control Tech*. Retrieved 3/7/2017. http://www.pctonline.com/article/pct1113-profits-mosquito-control/

Bonds, J. 2012. Ultra-low-volume space sprays in mosquito control: a critical review. *Med Vet Entomol* 26: 121-130.

[CDC] Centers for Disease Control and Prevention. 2016. West Nile Virus – Final Cumulative Maps and Data for 1999-2015. https://www.cdc.gov/westnile/statsmaps/cummapsdata.html

Chino-Cantor A, Sánchez-Arroyo H, Ortega-Arenas LD, & Castro-Hernández E. 2014. Insecticide Susceptibility of *Aedes aegypti* L.(Diptera: Culicidae) in Guerrero, Mexico. *Southwestern Entomol*, 39(3), 601-612.

Chism D, and Apperson C. 2003. Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosm by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. *Med Vet Entomol* 17: 211-220.

Cioto A, Kramer L. 2013. Vector-Virus Interactions and Transmission Dynamics of West Nile Virus. *Viruses* 5:3021-3047.

Day JF, Curtis GA. 1993. Annual emergence patterns of Culex nigripalpus females before, during and after a widespread St. Louis encephalitis epidemic in south Florida. *J Am Mosq Control Assoc* 9(3):249-255.

Day J. 2001. Predicting St. Louis encephalitis virus epidemics: Lessons from recent, and not so recent, outbreaks. *Ann Rev Entomol* 46: 111-125.

Devine G, Perea E, Killeen G, Stancil J, Clark S, Morrison A. 2009. Using adult mosquitoes to transfer insecticides to *Aedes aegypti* larval habitats. *PNAS* 106(28): 11530-11534.

Dick G, Kitchen S, Haddow A. 1952. Zika virus (I). Isolations and serological specificity. Trans Royal Soc Trop Med Hygiene 46(5):509-520.

El-Shazly M, Rafaie B. 2002. Larvicidal effect of the juvenile hormone mimic pyriproxyfen on *Culex pipiens*. *J Am Mosq Control Assoc* 18(4):321-328.

Farajollahi A, Fonseca DM, Kramer LD, Marm KA. 2011. “Bird biting” mosquitoes and human disease: A review of the role of *Culex pipiens* complex mosquitoes in epidemiology. *Infect, Gen, and Evol* 11:1577–1585.

Grard G, Caron M, Mombo I, Nkoghe D, Mboui Ondo S, Jiolle D, Fontenille D, Paupy C, Leroy EM. 2014. Zika Virus in Gabon (Central Africa) – 2007: A New Threat from Aedes albopictus? (*Aedes albopictus* Transmission of ZIKAV Virus, Gabon). *PLOS NegTrop Dis* 8(2):E2681.

Itoh T. 1994. Utilization of bloodfed females of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator, pyriproxyfen to larval habitats. *Trop Med* 36(4):243-248.

Juliano S, Lounibos L. 2005. Ecology and Invasive Mosquitoes: Effects on Resident Species and on Human Health. *Ecol Letters* 8:558-574.

Krow-Lucal E, Lindsey N, Lehman J, Fischer M, Staples J. 2017. West Nile Virus and Other Nationally Notifiable Arboviral Diseases – United States, 2015. US Department of Health and Human Services/Centers for Disease Control and Prevention. *Morbid Mort Weekly Rep* 66(2): 51-55.

Kuno G, Change G, Tsuchiya K, Karabatsos N, Cropp C. 1998. Phylogeny of the genus Flavivirus. *J of Virol* 72: 73-83.

LaDeau S, Leisnham P, Biehler D, Bodner D. 2013. Higher mosquito production in low-income neighborhoods of Baltimore and Washington, DC: Understanding ecological drivers and mosquito-borne disease risk in temperate cities. *Intern J Environ Research Pub Health* 10(4):1505-26 http://ezproxy.uky.edu/login?url=http://search.proquest.com.ezproxy.uky.edu/docview/1355951044?accountid=11836 (accessed August 7, 2016).

Ledesma N, Harrington L. 2011. Mosquito Vectors of Dog Heartworm in the United States: Vector Status and Factors Influencing Transmission Efficiency. *Elsevier: Topics in Compan Animal Med* 26(4):178-185.

Li C, Guo X, Dong Y, Wang G, Zheng Z, Zhao T. 2014. Evaluation of vegetation treatment with lambda-cyhalothrin to control *Aedes vexans* and *Simulium* maculatum in northern China. *J Am Mosq Control Assoc* 30(3):231-233.

Loftgren C. 1974. Recent developments in methods of mosquito control. *Bulletin of the World Health Organization* 50:323-328.

Madden A, Schroeder H, Lindquist A. 1947. Residual spray applications to salt-marsh and jungle vegetation for control of mosquitoes. *J Econ Entomol* 40:119-123.

Manore C, Ostfeld R, Agusto F, Gaff H, LaDeau L. 2017. Defining the Risk of Zika and Chikungunya Virus Transmission in Human Population Centers of the Eastern Unites States. *PLOS NegTrop Dis* 11(1):e0005255.doi:10.1371/journal.pntd.0005255.

Molaei G, Andreadis T. 2006. Identification of Avian- and Mammalian-Derived Bloodmeals in *Aedes vexans* and *Culiseta melanura* (Diptera: Culicidae) and Its Implication for West Nile Virus Transmission in Connecticut, U.S.A. *J Med Entomol* 43(5):1088-1093.

Moore C, Francy D, Eliason D, Monath T. 1988. *Aedes albopictus* in the United States: Rapid Spread of a Potential Disease Vector. *J Am Mosq Control Assoc* 4(3): 356-361.

Monaghan A, Morin C, Steinhoff D, Wilhelmi O, Hayden M, Quatrochi D, Reiskind M, Lloyd A, Smith K, Schmidt C, Scalf P, Ernst K. 2016. On the Seasonal Occurrence and Abundance of the Zika Virus Vector Mosquito Aedes Aegypti in the Contiguous United States. *PLOS Currents* 16(8): 1-24.

Moudy R, Meola M, Morin L, Ebel G, Kramer L. 2007. A Newly Emergent Genotype of West Nile Virus Is Transmitted Earlier and More Efficiently by *Culex* Mosquitoes. *Amer J Trop Med Hygiene* 77(2): 365-370.

Muzari O, Adamczyk R, Davis J, Ritchie S, Devine G. 2014. Residual effectiveness of λ-cyhalothrin harbourage sprays against foliage-resting mosquitoes in North Queensland. *J Med Entomol* 51(2):444-449.

N’Guessan R, Corbel V, Akogbéto M, Rowland M. 2007. Reduced Efficacy of Insecticide-treated Nets and Indoor Residual Spraying for Malaria Control in Pyrethroid Resistance Area, Benin. *Emerg Infect Dis* 13(2): 199. https://dx.doi.org/10.3201/eid1302.060631.

Pant C, Mathis H, Nelson M, Phanthumachinda B. 1974. A large-scale field trial of ultra-low-volume fenitrothion applied by a portable mist blower for the control of *Aedes aegypti*. *Bull World Health Org* 51:409-415.

"Public-Health Pesticide Applicator Training Manual - Mosquitoes". *Entomology.ifas.ufl.edu*. N.p., 2017. Web. 22 Feb. 2017.

Püntener, W. 1981. Manual for field trials in plant protection second edition. Agricultural Division, Ciba-Geigy Limited.

Reinert, J. F. 2000. New classification of the composite genus *Aedes* (Diptera: Culicidae: Aedini), elevation of *Ochlerotatus* to generin rank, reclassification of the other subgenera, and notes on certain subgenera and species. *J Am Mosq Control Assoc*16: 175 – 189.

Reinert J, Harbach R, Kitching I. 2004. Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. *Zoo J Linnaen Soc* 142 (3): 289-368. doi:10.1111/j.1096-3642.2004.00144.x

Reisen WK. 2016. Update on Journal Policy of Aedine Mosquito Genera and Subgenera. *J Med Entomol* 53(2): 249. doi: 10.1093/jme/tjv169

Sardelis M R, Turell MJ. 2001. *Ochlerotatus japonicus* in Frederick County, Maryland: discovery, distribution, and vector competence for West Nile virus. *J Am mosq control assoc* 17(2):137-141.

Service M. 2008. Medical Entomology for Students. New York, NY. *Cambridge University Press*.

Sprenger D, Wuithiranyagool T. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *J Amer Mosq Control Assoc* 2: 217-219.

Suman D, Wang Y, Dong L, Gaugler R. 2013 Effects of Larval Habitat Substrate on Pyriproxyfen Efficacy Against *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol* 50(6):1261-1266. doi: 10.1603/ME13068

Suman D, Farajollahi A, Healy S, Williams G, Wang Y, Shoeler G, Gaugler R. 2014. Point-source and area-wide field studies of pyriproxyfen autodissemination against urban container-inhabiting mosquitoes. *Acta Tropica,* 135:96-103.

Thacker J. 2002. An Introduction to Arthropod Pest Control. New York, NY. *Cambridge University Press.*

Trout R, Brown G, Potter M, Hubbard J. 2007. Efficacy of two pyrethroid insecticides applied as barrier treatments for managing mosquito (Diptera: Culicidae) populations in suburban residential properties. *J Med Entomol* 44(3):470-477.

Voelker R. 2014. West Nile Virus Leading Cause of Neuroinvasive Arboviral Disease. *JAMA: J Amer Med Assoc* 312(7): 689.

Wilkerson RC, Linton Y-M, Fonseca DM, Schultz TR, Price DC, Strickman DA 2015. Making Mosquito Taxonomy Useful: A Stable Classification of Tribe Aedini that Balances Utility with Current Knowledge of Evolutionary Relationships. *PLoS ONE 10*(7): e0133602. doi:10.1371/journal.pone.0133602

Wong P-S, Li M, Chong C-S, Ng L-C, Tan C-H. 2013. *Aedes* (Stegomyia) *albopictus* (Skuse): A Potential Vector of Zika Virus in Singapore. *PLOS Neg Trop Dis.* 7(8): e2348. doi:10.1371/journal.pntd.0002348.

Zhu Z, Chan J, F-W, Tee K-M, Choi G, K-Y Lau, S, K-P, Woo P, C-Y, Tse H, Yuen K-Y. 2016. Comparative genomic analysis of pre-epidemic and epidemic Zika virus strains for virological factors potentially associated with the rapidly expanding epidemic. *Emerg Microb Infect* 5: e22; doi:10.1038/emi.2016.48

VITA

Andrea Glenn Skiles

Educational institutions attended and degrees awarded

Bachelor of Arts, Ohio Wesleyan University, Delaware, OH: Bachelor of Arts: Biology; May 2013

Professional positions held

2016-present Senior Laboratory Technician**,** Department of Entomology**,** University of Kentucky, Lexington, KY

Fall 2014 Research Technician, Department of Forest Resources, University of Minnesota, Cloquet, MN

Summer 2014 Research Technician, School of Natural Resources, University of Nebraska-Lincoln, Lynch, NE

Spring 2014 Field Technician, School of Environment and Natural Resources, The Ohio State University, McArthur, OH

2013 Research Technician, Department of Forest Resources, University of Minnesota, Grand Rapids, MN

Professional presentations

A. Glenn Skiles, Nicola T. Gallagher, and Grayson C. Brown. (2017). The use of a truck-mounted sprayer for the application of a residual barrier treatment using pyriproxyfen, both alone and in combination with lambda-cyhalothrin, for the improved suppression of mosquitoes. American Mosquito Control Association Annual Meeting, San Diego, CA.

A. Glenn Skiles, Kyndall C. Dye, Nicola T. Gallagher, and Grayson C. Brown. (2016). Increasing the effective duration of mosquito suppression on a small spatial scale (less than 0.25 ha). Oral Presentation. XXV International Congress of Entomology, Orlando, Florida, USA

A. Glenn Skiles. (2016). Preparing for Zika: Mosquito Control Efforts in a Kentucky Public Health District. Extension Seminar presented to Green River District Health Department employees and constituents, Owensboro, KY. Invited talk.

Grayson C. Brown, **A. Glenn Skiles**, Kyndall C. Dye. (2016). New developments in backyard mosquito control and their relation to mosquito-borne disease. National Conference of Urban Entomologists, Albuquerque, NM. Invited talk.

A. Glenn Skiles, Kyndall C. Dye, Nicola T. Gallagher and Grayson C. Brown. (2016). Adding an IGR to mosquito barrier treatments to increase residual effectiveness in suburban backyards. Oral Presentation. American Mosquito Control Association Annual Meeting, Savannah, GA

A. Glenn Skiles, Kyndall C. Dye, Nicola T. Gallagher and Grayson C. Brown. (2015). Increasing the Effective Duration of a Pyrethroid Insecticide in Suburban Mosquito Management. Poster Presentation. 63rd Annual Meeting of the Entomological Society of America, Minneapolis, MN

Publications

Grayson C. Brown, Kyndall C. Dye, **A. Glenn Skiles**. (2016). Are You Prepared to Battle the Zika Virus? Healthy Living Made Simple. July/August issue.

Professional Awards

Washington Conference Fellowships Travel Stipend. (March 2017). American Mosquito Control Association, 19th Annual Washington Conference. Washington, DC. $1,000.

Young Professional of the Month. (December 2016). American Mosquito Control Association. University of Kentucky.

Young Professionals Travel Stipend. (April 2016). American Mosquito Control Association, Annual Meeting. Savannah, GA. $1,000.

TEACHING EXPERIENCE

ENT 300. Fall, 2016. Teaching Assistant for General Entomology. Professor – Dr. Jen White.

Mosquito Identification Course. June, 2016. Identification of mosquitoes common in Kentucky. Louisville Metro Department of Public Health.

Professional memberships

American Mosquito Control Association	August, 2015 – Present
Entomological Society of America	August, 2015 – Present
H. Garman Entomology Club	January, 2015 – Present

Andrea Glenn Skiles